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6447

Combined Action of Ethyl Urethane and Sodium Thiocyanate on the Living Cell.

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The alleged ability of sodium rhodanate (thiocyanate) to peptize heat-coagulated egg albumin led Bancroft and Rutzler[1] to elaborate a general theory, based upon an early observation of Claude Bernard,[2] that anesthesia is the result of a reversible coagulation of the colloids of the brain-cells, and that the action of narcotics in general can be antagonized by peptizing agents such as NaCNS and NaI. Their principle has been so extended in its application that now such pathological conditions as drug addiction,[3] histamine poisoning,[4] and insanity[5, 6] are held by them to result from disturbances in the colloidal state of the nervous system.

[1] Bancroft, W. D., and Rutzler, J. E., Jr., *J. Phys. Chem.*, 1931, **35**, 144, 215, 1185, 3036.

[2] Bernard, Claude, *Union Med. Paris*, 1869, **8**, 109.

[3] Bancroft, W. D., and Rutzler, J. E., Jr., *J. Phys. Chem.*, 1932, **36**, 1521, 2011.

[4] Bancroft, W. D., and Rutzler, J. E., Jr., *J. Phys. Chem.*, 1931, **35**, 3189.

[5] Bancroft, W. D., and Richter, G. H., *J. Phys. Chem.*, 1931, **35**, 1606.

[6] Bancroft, W. D., and Richter, G. H., *Proc. Nat. Acad. Sci.*, 1931, **17**, 294.

The action of NaCNS as a narcotic antagonist was studied, to determine whether the action postulated for it could be observed in the case of a simple cell. The material was the egg of *Arbacia punctulata*, selected for its convenient size, ready availability, and because of the great amount of information amassed concerning it.[7]

Preliminary determinations were made to determine the toxic and lethal effects of different concentrations of ethyl urethane and sodium thiocyanate. In all cases freshly shed eggs of a single female were selected, using the criteria suggested by Just[8] for obtaining normal eggs. The eggs were washed twice to remove the perivisceral fluid, pipetted into sea-water solution, beakers covered, and after requisite time, washed and inseminated in sea-water. Controls were run for each determination. The solutions were made from purest reagents obtainable, with sea-water acting as natural buffer. The criterion of viability was the development of gastrulae and pluteii. When striking abnormalities were observed in the larvae the concentration was designated as toxic; when development was inhibited, as lethal.

The following table summarizes the results for the narcotic:

Concentration ethyl urethane	Effect of ½ hr. exposure	Effect of 2 hr. exposure
%		
0	None	None
1	"	
1.5	Slightly toxic	Toxic
2	Toxic	Lethal
3	Lethal	"

This indicates a value of 1.5% for the slightly toxic dose, corresponding with Heilbrunn's value[9] for the anesthetic concentration. The value of 3% for the lethal dosage is the same as his. There was no need to consider the osmotic values of the solution since the narcotic penetrates immediately into the cell.

The results for NaCNS were as follows:

All runs showed similar results. The values between 3% and 4% varied, being always toxic and sometimes lethal. The molarity values quoted in the table are for the whole solution, sea-water plus NaCNS, with the molar concentration of sea-water being taken as equivalent to 0.52M NaCl. The values of 3, 3.5, and 4% (of which the value in the table is an average result) were all made

[7] Harvey, E. Newton, *Biological Bulletin*, 1932, **62**, No. 2.

[8] Just, E. E., *Protoplasma*, 1928, **5**, 97.

[9] Heilbrunn, L. V., The Colloid Chemistry of Protoplasm, Borntraeger, 1928.

Concentration NaCNS	Effect of ½ hr. exposure	Effect of 2 hr. exposure
%		
0	None	None
2.1 (.78M)	"	Slightly toxic
3.5 (.52M)	Toxic	Lethal
5	Lethal	"

isosmotic with the eggs, the necessary amount of distilled water being added.

According to Bancroft and Rutzler the effect of the narcotic should be antagonized by some concentration of NaCNS capable of penetrating into the cell and exerting an effect upon the cytoplasmic colloids; the peptizing action of the salt should compensate for the coagulative action of the narcotic. To test this assumption concentrations of ethyl urethane were chosen which were known to coagulate the protoplasm; Heilbrunn's work[9] showed these to be our toxic or lethal values. It appeared desirable to antagonize these narcotic values with both a harmless and a toxic concentration of the salt. In results to be reported later it will be shown that NaCNS, like most strongly ionized salts, penetrates only very slowly into the cell, but that the minute quantity which does gain access is sufficient to bring about a definite change in the colloidal condition of the cytoplasm. Such a viscosity change can be demonstrated after exposure to the 2.1% or 3.5% NaCNS within one-half hour. Thus it did not appear essential to use a wide range of concentrations.

When eggs were placed in ethyl urethane plus NaCNS in seawater the following results were obtained:

Concentration ethyl urethane + NaCNS		Effect of ½ hr. exposure	Effect of 2 hr. exposure
%	%		
0	0	None	None
2	3.5	Lethal	Lethal
2	2.1	"	"

The results show no antagonism but an additive toxic effect upon treatment with the two reagents. For the half-hour period the 3.5% NaCNS and the 2% ethyl urethane were both toxic when used by themselves, but their combined effect was lethal. More convincing evidence comes from the runs with the other concentrations: 2% urethane is toxic, while 2.1% NaCNS is harmless, whereas together they are definitely lethal. It is to be remembered that the cumulative effect cannot be attributed to the increased os-

motic pressure theoretically obtainable with the 2 reagents in solution together, the narcotic being osmotically inactive due to its rapid penetration and the immediate attainment of its equilibrium.

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Relation of Plasma Volume to Plasma Protein Concentration.

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It was the purpose of this study to determine the relationship of plasma protein concentration to the plasma volume of non-edematous dogs. The value of plasma albumin determinations in studying fluid exchange has been amply demonstrated. It was consequently determined to perform simultaneous analyses of plasma albumin and globulin concentrations and of plasma volume in dogs that were undergoing certain experimental procedures. The method of Koch and McMeekin[1] was used for determining the plasma protein levels of the first 2 dogs and a micro-kjeldahl procedure involving distillation into N/70 H_2SO_4 and subsequent titration with N/20 alkali was used for the other 2 dogs. The circulating plasma volumes of the first 2 dogs were kindly determined for me by Mr. John Morcan, who used the brilliant vital red technique as described by Whipple and coworkers.[2] Those of the other 2 dogs were performed by the author, utilizing the spectrophotometric procedure for analyzing brilliant vital red described by Clarke and Graff.[3]

Changes in the plasma albumin concentration proved to be the most significant ones and consequently they alone will be mentioned in this report.

Two dogs in the anemia colony in this laboratory were studied weekly over a period of 2-3 months. In one of them, both the plasma volume and the plasma albumin concentration remained fairly constant. In the other, as the plasma albumin concentration decreased, the plasma volume decreased. As the plasma albumin concentration increased the plasma volume increased.

[1] Koch, F. C., and McMeekin, T. L., *J. Am. Chem. Soc.*, 1924, **46**, 2066.

[2] Hooper, C. W., Smith, H. P., Belt, A. E., and Whipple, G. H., *Am. J. Physiol.*, 1920, **51**, 205.

[3] Graff, S., and Clarke, H. T., *Arch. Int. Med.*, 1931, **48**, 808.

Two other dogs (not anemic*) were placed upon a low protein diet developed by Weech and Goettsch[4] and studied weekly for about 2 months. This diet consisted mainly of carrots and contained essential salts and apparently all of the recognized vitamins. One of these dogs showed small changes in plasma volume that were paralleled as above by changes in the plasma albumin concentration. The other dog showed large changes in plasma volume which paralleled changes in the plasma albumin concentration.

It was found that whole blood volume also varied directly with the plasma albumin concentration. The following table is a protocol of the results obtained with one of the dogs in the anemia colony.

TABLE I.

Day	Plasma Albumin gm./100 cc.	Plasma Volume cc.	Blood Volume cc.
1	3.82	1155	1414
10	4.92	1178	1435
17		1141	1498
24	4.30	1115	1450
31		1003	1358
38	3.42	822	1136
44	2.14	691	1073
56	3.42	961	1260
63	3.63	1018	1260
71	4.17	1074	1301

From these experiments it appears that there is a rather constant and direct relationship between the plasma albumin concentration and the plasma volume, which can be explained adequately on the basis of the Starling conception of fluid exchange, for according to this theory a rise in plasma colloid osmotic pressure (other factors remaining constant) will attract fluid into the blood and a fall in the plasma colloid osmotic pressure will permit filtration of fluid out of the blood. It is therefore concluded that the concentration of plasma albumin may be one factor concerned in the regulation of plasma volume.

* This experiment was conducted at the Babies' Hospital in New York, N. Y. It is a pleasure to acknowledge my indebtedness to Dr. A. A. Weech and his associates for encouragement and cooperation; to Dr. S. Graff of the College of Physicians and Surgeons for teaching me the spectrophotometric technique for determining plasma volume.

[4] Weech, A. A., Snelling, C. E., and Goettsch, E., *Am. J. Dis. Child.*, 1932, **44**, 657.

Southern Section.

Birmingham, Alabama, November 16, 1932.

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Observations Upon Complement Fixation in Experimental Amebiasis in Dogs.*

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The writer[1] demonstrated the presence of complement fixing bodies in the blood serum of individuals infected with *Endamoeba histolytica* and subsequently[2, 3, 4, 5, 6] reported the results obtained with a complement fixation test in cases of human amebiasis and in normal individuals, together with the technique of the test. These observations have been confirmed by Spector,[7] Fernandez,[8] Heathman,[9] and Sherwood and Heathman.[10]

The observations here reported were undertaken to throw further light upon the phenomenon of complement fixation in amebiasis, using the dog, an animal that Faust[11] has shown is easily infected with *Endamoeba histolytica* and in which the lesions produced by this parasite are comparable to those occurring in intestinal amebiasis in man. Alcoholic extracts of cultures of *Endamoeba histolytica* were used as antigens.

Tests were made upon 29 dogs infected with *Endamoeba histo-*

* Aided by a grant from the David Trautman Schwartz Fund.

1 Craig, C. F., *Am. J. Trop. Med.*, 1927, **7**, 225.
2 Craig, C. F., *Proc. Nat. Acad. Sci.*, 1928, **14**, 520.
3 Craig, C. F., *Am. J. Trop. Med.*, 1928, **8**, 29.
4 Craig, C. F., *Am. J. Trop. Med.*, 1929, **9**, 277.
5 Craig, C. F., *J. Am. Med. Assn.*, 1930, **95**, 10.
6 Craig, C. F., *Ann. Int. Med.*, 1931, **5**, 170.
7 Spector, B. K., *J. Prev. Med.*, 1932, **6**, 117.
8 Fernandez, P. E., *Am. J. Hyg.*, 1932, **15**, 785.
9 Heathman, L., *Am. J. Hyg.*, 1932, **16**, 97.
10 Sherwood, N. P., and Heathman, L., *Am. J. Hyg.*, 1932, **16**, 124.
11 Faust, E. C., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 908.

lytica and in all but one (96.5%) a positive complement fixation reaction developed in the blood serum. In the dog giving a negative reaction death occurred 7 days after colonic inoculation from a very severe amebic colitis. Of the dogs giving a positive reaction, 21 (75%) gave a 4+ reaction; 3 (10.7%) gave a 3+ reaction; 2 (7.1%) gave a 2+ reaction; and 2 (7.1%) gave a 1+ reaction.

Of the 28 dogs giving a positive reaction 22 died and 5 were sacrificed, 3 while still suffering from intestinal amebiasis while 2 had recovered from the infection. Of the 22 dogs that died, all showed typical amebic ulcerations in the colon and in all motile forms of *Endamoeba histolytica* were found in the lesions. The 3 dogs killed while still suffering from the infection showed both healed and active amebic lesions in the colon and motile *Endamoeba histolytica* were present in the active lesions. In the 2 dogs sacrificed after recovery, healed amebic ulcerations were found in the colon but the gut was negative for *Endamoeba histolytica.* Thus, the positive results obtained with the test in these dogs were confirmed in 100% of the cases by the pathological lesions found at autopsy and in all but the 2 recovered cases, by the presence of motile *Endamoeba histolytica* in the lesions. In addition, this parasite was found in the feces of every dog giving a positive complement fixation test.

The *time of appearance of the reaction* in the infected dogs varied between 3 days and 14 days after inoculation in those animals tested before inoculation. In one dog a 2+ reaction was obtained 3 days after inoculation; in 3, a 4+ reaction 4 days after inoculation; in 1, a 4+ reaction in 5 days; in 1, a 3+ reaction in 5 days; in 1, a 4+ reaction in 9 days; in 5, a 4+ reaction in 10 days; in 3, a 3+ reaction in 12 days; in 1, a 1+ reaction in 13 days; in 1, a 2+ reaction in 14 days; and in 1, a 4+ reaction in 14 days after inoculation.

The very early appearance of the reaction in some dogs was remarkable but no more so than the extent and character of the amebic lesions observed in such animals at autopsy. In Dog 125, giving a double-plus reaction 3 days after inoculation death occurred upon the same day and, at autopsy, wide-spread superficial amebic ulcerations were found in the appendix and colon and motile trophozoites of *Endamoeba histolytica* were present in the lesions. In Dog 126, a 3+ reaction appeared upon the 5th day after inoculation and the animal died upon the same day. At autopsy, amebic ulcerations were numerous throughout the colon and motile amebae were found in the lesions; while in Dog 143, giving a 4+ reaction

4 days after inoculation, and dying upon the 8th day after inoculation, amebic ulcers were numerous throughout the colon, especially in the rectum, and motile trophozoites of *Endamoeba histolytica* were present in the lesions. In these very susceptible dogs the reaction appears very promptly and the invasion of the tissues by the amebae, with the production of very severe lesions, is surprisingly rapid and extensive.

The gradual disappearance of the positive reaction was noted in the dogs that recovered spontaneously or after being placed upon a liver diet which resulted in the disappearance of the symptoms of infection.

The results of the complement fixation test upon 42 dogs free from infection with *Endamoeba histolytica* used as controls were uniformly negative and at autopsy these animals did not show any evidence of amebic infection.

6450

Studies Upon the Filterable and Non-Filterable State in the Tunnicliff Coccus of Measles.

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From the Department of Pathology and Bacteriology, Tulane Medical School.

This communication reports the results of tests upon the differences in filtrability of the Tunnicliff coccus of measles under *in vivo* and *in vitro* growth conditions. The question whether microorganisms ordinarily regarded as non-filtrable can become filtrable and vice versa, under special conditions of growth, is of more than academic interest. Kendall,[1] with the use of his "K" medium, claims that it is relatively easy to cause non-filtrable organisms to become filtrable and filtrable to become microscopically visible and non-filtrable.

Different environmental conditions cause some microorganisms to undergo rather marked changes in size and shape of the individual cell. Particularly is this true for certain parasitic varieties capable of cultivation *in vitro*, where they are distinctly larger and often differently shaped than they are *in vivo*. Adaptation to the artificial environment is the accepted explanation for the morphological variation. The reaction of the culture medium, quality of nutritives,

[1] Kendall, A. I., *Northwestern Univ. Bull.*, 1931, **32**, No. 8.

absence of the living host influence and the osmotic pressure are undoubtedly responsible for this *in vitro* metamorphosis.

Experiments. Full-grown, healthy guinea pigs were intracardially injected with 1 cc. quantities of a heavy saline suspension from a 24-hour blood-agar growth of pure cultures of the Tunnicliff coccus of measles. The cultures used for animal inoculation were isolated in 1926 by Duval and Hibbard[2] directly from the blood of human measles upon a modified Noguchi plasma medium. The morphology was that of a Gram positive diplococcus corresponding in size to the ordinary pneumococcus.

Another series of animals was inoculated intracardially with 5 cc. of the Berkefeld "N" filtrate from 24-hour growth of the culture. From the filtrate tested for sterility upon a variety of media, no growth of any kind was obtained. Likewise, there were no filtrable forms present in the filtrate since the animals inoculated failed to react and nothing was recovered from their cultured blood.

Three days after inoculation the animals were sacrificed and the hearts' blood collected under sterile conditions for study. Parts of the collected guinea pig blood were filtered through the Berkefeld "N" filter and planted upon a variety of media including the "K" medium of Kendall. Parts of the unfiltered blood were likewise cultured upon similar media. Regularly, though with considerable difficulty and then only upon special media, was the coccus of measles recovered from the Berkefeld "N" filtered guinea pigs' blood. Growth in the coccal form was obtained earlier upon "K" medium of pH 8.4-7.2 than any other. Upon this medium the coccus culture was established in 2 to 3 days.

Comments. We believe that the results are further evidence that the Tunnicliff coccus of measles *in vivo* (man, guinea pig, rabbit, monkey) is extremely minute (virus form) and readily filtrable through the "N" Berkefeld filter. On the other hand, in adaptation to an *in vitro* environment it becomes the familiar coccal form which is not filtrable. Of further significance is the fact that in repeated passage of the culture through animals there is the same remarkable metamorphosis.

Experiments with culture filtrate prove conclusively that there is no separate filtrable virus in symbiosis or otherwise with the Tunnicliff coccus culture. Likewise, tests show that the culture *in vitro* does not contain a filtrable and non-filtrable form of the same micro-

[2] Hibbard, R. J., and Duval, C. W., PROC. SOC. EXP. BIOL. AND MED., 1926, **23**, 853.

organism (Tunnicliff coccus). In view of Hadley's[3] claim that certain cultures of microorganisms possess simultaneously, filtrable and non-filtrable forms, it was considered a possibility in the case of the Tunnicliff coccus but was unsupported by experimentation.

Special media have nothing to do with causing the Tunnicliff coccus to become filtrable or reinducing it subsequently to become non-filtrable. The interchangeable metamorphosis is determined by natural environmental factors (living tissue) on the one, and by artificial growth conditions on the other hand.

Finally Tunnicliff's coccus of measles is capable of existing in two states, filtrable *(in vivo)* and non-filtrable *(in vitro)*.

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Eosin-Light Injury of Erythrocytes. 1. The Influence of Certain Ions.*

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To investigate the problem of photo sensitization in pellagrins, need has been felt for quantitative *in vitro* methods. The observations here described were made with the hope of aiding in the development of such methods.

As a sensitizing agent yellow eosin (National Analine and Chemical Company) has been used. The light source was a nitrogen-filled tungsten filament lamp of 1500 watts, operated at 115 volts by alternating current. The suspension of erythrocytes was contained throughout exposure to light and measurement of degree of hemolysis in standard Pyrex test tubes of 16 mm. outside diameter with walls 1.5 mm. thick. Equal exposure of each tube was insured by placing the tubes in a device to be described in detail elsewhere. This apparatus moved tubes in a circle 30 cm. in diameter about the light source at the approximate center, while the tubes were immersed in a constant temperature waterbath at 37.5° C. and received light through plane windows of plate glass 3 mm. thick. The layer of tap water between the tube and the plate glass was 2 mm. where the tube's circumference came closest to the window. Adequate provision for aeration and maintenance of suspension was

[3] Hadley, P., *J. Infect. Dis.*, 1927, **40**, 301.

* Aided by grants from the David Trautmann Schwartz Research Fund.

provided. Human blood from finger prick was allowed to fall into 0.85% NaCl solution made sufficiently alkaline to give a pink color with phenol red by the addition of 0.1 N NaOH and washed by centrifugation 3 times in specimens of the same solution. This cell suspension was added in 0.2 cc. quantities, measured from pipettes of the between-marks type into 5 cc. quantities of the various solutions which were under study. The final concentration of erythrocytes was about 15,000 per mm^3; it was kept close to this figure by adjusting the concentration of the heavy suspension as was indicated by photometer readings of the trial dilutions. All solutions were isotonic as determined by the hematocrit method.

In the present communication hemolysis is used as the criterion of erythrocyte injury. The degree of hemolysis was estimated by measuring the light transmission of the cell suspension. This principle was introduced by Ponder.[1] A photoelectric tube photometer, which measures light transmission of solutions and suspensions in 16x155 mm. test tubes, was used. This instrument will be described in detail elsewhere. Properly calibrated it has proved highly satisfactory for following the progress of hemolysis.

In order to determine the effect of hydrogen ion concentration upon eosin-light hemolysis, buffer solutions of mixtures of primary and secondary potassium phosphate were prepared containing 1-200,000 eosin. Fig. 1 shows the results of one experiment in which the exposure to light was for 40 minutes. It will be noted that within the ranges of hydrogen ion concentration studied there was a tendency for hemolysis to become less rapid as hydrogen ion concentration diminishes. Repetition of this experiment with different concentration of eosin and different time of exposure has given similar results.

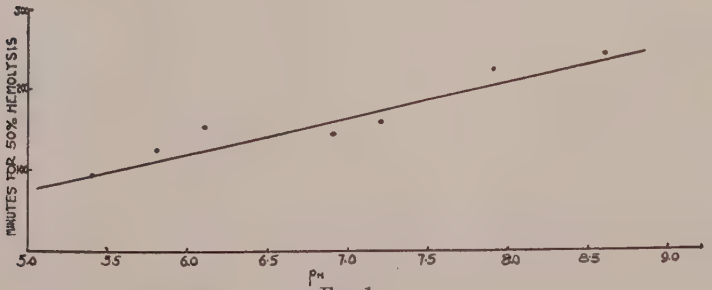

FIG. 1.

[1] Ponder, E., *Proc. Roy. Soc.*, 1923, **95**, 382.

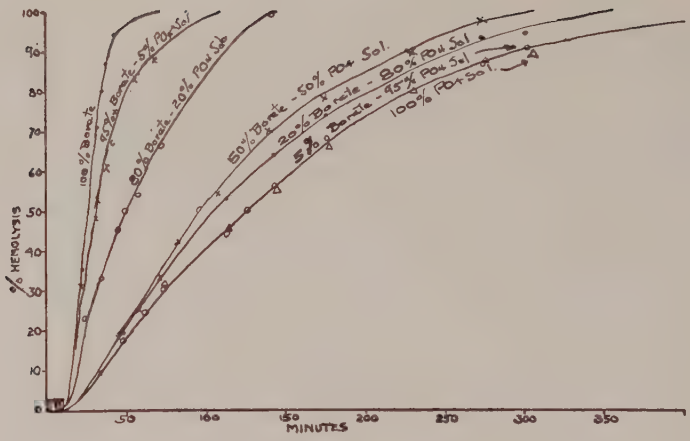

FIG. 2.

A buffer solution containing sodium borate and hydrochloric acid of pH 7.5 was prepared. Its effect on eosin-light hemolysis was compared with that of a potassium phosphate buffer of the same pH, each with an eosin concentration of 1-10,000. The influence on hemolysis of the 2 solutions and various mixtures of the 2 is shown for one experiment in Fig. 2. Borate served to accelerate and phosphate to retard hemolysis. The velocity of hemolysis was not demonstrably different in solutions in which the sole buffer salt was potassium phosphate than those containing 5 volumes per hundred of borate buffer solution. Had light dosage been sufficient to have caused more rapid hemolysis in these particular solutions a difference might have been demonstrated.

In another series of experiments each tube contained 2 cc. of sodium borate-HCl buffer, 3 cc. of solution of neutral salts and the usual 0.2 cc. of erythrocyte suspension. The borate solution had an eosin content sufficient to give a final concentration of 1-50,000. The results of one such experiment which may be accepted as typical are shown in part in Fig. 3. The neutral salts thus tested were: KCl, NaCl, and Na_2SO_4. Potassium phosphate was included in the forms of a buffer solution of pH 7.5. Solutions of $CaCl_2$, and $MgCl_2$ were mixed with solutions of NaCl so that the final concentration of calcium was 24 mg. per 100 cc. and magnesium 31 mg. per 100 cc. Hemolysis in the calcium and magnesium solutions was at a velocity not measurably different from that taking place when NaCl solution without either calcium or magnesium chloride was employed.

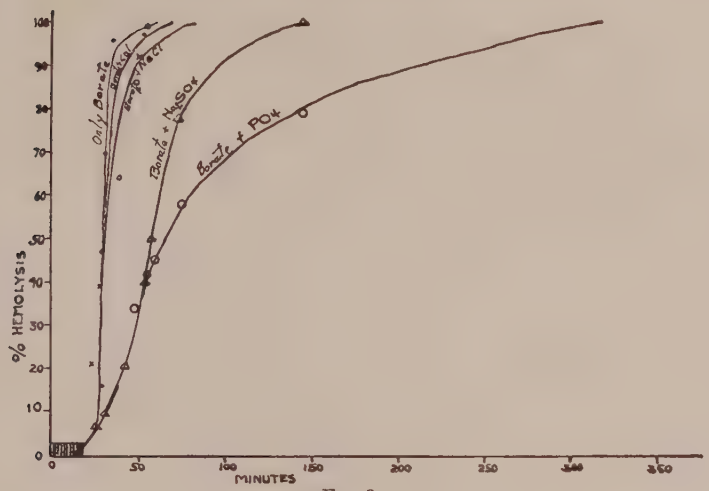

FIG. 3.

As control all suspensions in the various solutions studied containing eosin but unexposed to light were observed for hemolysis. In only one solution was hemolysis noted. That was in the phosphate buffer of pH 5.4. Proper correction in the velocity of hemolysis for the exposed specimen was made. Cell suspensions in the solutions under study containing no eosin were also exposed to light for the same duration as those containing eosin. In no instance was hemolysis observed.

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Infection of Kittens with Endamoeba histolytica by Direct Injection of Cultures into the Ileum.

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In the course of experiments to test the relative pathogenicity for kittens of a number of strains of *Endamoeba histolytica* in culture, rectal injection proved unsatisfactory because of the small percentage of kittens which became infected. The technique of rectal injection was simply to withhold food on the day of injection, and inject the sediment from a rich culture by means of a rubber-tipped

pipette which was inserted gently through the anus to a distance of about 3 inches. The kitten was then held head downward for a few minutes before being released. In one series the anus was sutured for 24 hours, without producing any better results. A preliminary enema, as used by Kessel[1] was not given because of the desire to avoid even microscopic trauma as far as possible and to avoid early evacuation of the injected material.

Feeding cysts by mouth produced no better results with the one strain with which the method was employed. This method was also impracticable because some strains did not form cysts in culture.

In considering a better route of infection, injection directly into the cecum after laparotomy, as practiced by Rees[2] was not adopted because it was not desirable to pass a needle directly through the wall of the cecum. It was felt that this might create a small lesion in which a few amoebae or bacteria might be left in withdrawing the needle. Faust's method[3] of inoculating dogs directly into the ileum through the anus was not feasible because the colon of the cat is acutely flexed in its upper third, and because there was too much danger of trauma.

It was therefore decided to make the injection directly into the ileum, thus allowing the amoebae to enter the large intestine as they normally would in oral inoculations with cysts. Kittens were anesthetized with ether, the abdomen was opened by an upper mid-line incision, the cecum, which often presented into the wound, was gently pulled outside and the culture injected into the ileum about 5 cm. above the ileocecal valve through a 20 gauge needle. A 5% solution of mercurochrome was applied to the point of injection as the needle was withdrawn to avoid soiling the peritoneum, and the abdomen closed by a continuous silk suture in the muscle and by skin clips. Laparotomy did not shorten the average duration of life of the kittens.

Table I shows a comparison of the results obtained by this method and by rectal injection. With all 5 strains of amoebae the incidence of infection was much higher in the series injected by ileum than in those injected by rectum. The average for all the strains was 54.8% infected by ileum against 21.6% by rectum. The greatest relative increase by ileum was in the strains giving the lowest incidence of infection by rectum. The increased incidence by ileum was obtained despite the fact that, except for the "Ware" strain, the

[1] Kessel, J. F., *Am. J. Hyg.*, 1928, **8**, 311.

[2] Rees, C. W., *Arch. Path.*, 1929, **7**, 1.

[3] Faust, E. C., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 908; *Porto Rico J. Pub. Health and Trop. Med.*, 1931, **6**, 391.

TABLE I.
Inoculation of Kittens with Cultures of *E. histolytica* by Rectum and by Ileum.

Strain of *E. histolytica*	Route of Inoculation	No. Kittens Inoculated	Kittens Developing Amoebic Colitis	
			No.	%
Daffo	Rectum (Not sutured)	20	1	5.0
	Ileum	38	12	31.6
Nelson	Rectum (Not sutured)	36	5	13.9
	Rectum (Sutured)	22	2	9.1
	Ileum	19	15	79.0
Ware	Rectum (Not sutured)	20	3	15.0
	Ileum	20	9	45.0
Cain	Rectum (Not sutured)	30	13	43.3
	Ileum	20	12	60.0
Keen	Rectum (Not sutured)	34	11	32.3
	Ileum	20	13	65.0
Total	Rectum	162	35	21.6
	Ileum	117	61	54.8

amoebae had been in culture a much longer time than they had been when the rectal work was done. Although the average age of the transplants of cultures used in the ileum work happened to be less than the average age of transplants used in the rectal work (we have found that younger transplants produce a higher incidence of infection) this does not invalidate our results, since 4 to 7 day cultures inoculated by ileum produced a considerably higher percentage of infection (45%) than did 1 to 3 day cultures by rectum (31%).

The lesions produced in the kittens injected by ileum did not differ essentially from those produced by rectal injection, except that in the kittens injected by ileum lesions were found more frequently involving the ileo-cecal valve and the cecum adjacent to it. In addition, 3 kittens injected by ileum showed slight lesions in the terminal ileum.

As a result of these observations we believe that injection into the ileum is much more valuable than injection by rectum in testing the pathogenicity for kittens of *E. histolytica* trophozoites in culture. Its only drawback is the necessity for surgical technique, but the uncertainty of giving the amoebae an opportunity to invade the tissues by the rectal route makes the more laborious procedure preferable.

Minnesota Section.

University of Minnesota, November 16, 1932.

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Some Characteristics of the Electro-Myograms of Quick Voluntary Muscle Contractions.

STARKE HATHAWAY. [Introduced by A. T. RASMUSSEN.]

From the Department of Psychology, University of Minnesota.

The electro-myograms making up the data from which these observations are reported were obtained from oscillographic records of amplified muscle action potentials. Ten adult human subjects were used. The action potentials were picked up by modified pore electrodes[1] which were inserted into the body of the muscle to be investigated. The active electrodes were insulated from all but a small area of the interior of the muscle, allowing fair isolation of antagonistic muscles. The amplified action potentials were photographed in a Westinghouse multi-element oscillograph. Action potentials could be simultaneously photographed from 2 different sources, usually the biceps and triceps brachii of the right arm.

A simple reaction time set-up was used. The subject was instructed to extend his forearm as quickly as possible following the disappearance of the glow of a low wattage neon bulb. The arm was supported horizontally on a rest allowing free movement about the elbow. The photographs included markers showing arm movement, time in 10 sigma intervals and a marker showing when the subject was given the ready signal and the stimulus. Reaction times were read to the first action potentials after the stimulus and to the first arm movement. The records show an average of about 56 sigma lapse between the first large action potential changes and the corresponding movement of the forearm. This time is fairly constant, varying in ordinary subjects by not more than ±8 sigma. There was no evidence of a preliminary tension in either the muscle which was to be used in the response or its antagonist preceding the stimulus and related to the warning signal.

[1] Adrian, E. D. and Bronk, D. W., *J. Physiol.*, 1928, **67**, 119.

Almost invariably action potentials in the reacting muscle were accompanied by corresponding ones in its antagonist. These antagonistic potentials might or might not correspond in time with the start of the reaction though it was usually within 10 sigma of being the same.

Wholly unexpectedly about 40% of all the records had an early volley of small discharges which appeared in as little as 25 sigma following the stimulus. The records from 3 of the subjects showed none of these early discharges and those from the remainder showed varying numbers, never, however, 100%. These early discharges, which were not accompanied by gross movement of the arm, occurred from 110 to 160 sigma earlier than the arm movement of the response. In the responding muscle they become larger, which augmentation was followed in 58 sigma (as above) by the first arm movement. These pre-response discharges were found in not only the responding muscle but also in its antagonist, in the triceps and biceps brachii of the contralateral arm which did not grossly move at all, and in the homolateral gastrocnemius. They are therefore quite widespread in their appeaarnce.

No complete theory can as yet be formulated in explanation of these pre-response discharges. The times of their appearance correspond roughly to the times for simple reflexes. It is possible that they originate in lower levels neurologically and so point to another relationship of the voluntary to the involuntary reaction. Further investigation is necessary in order to formulate more clearly the possibilities.

6454

Solubility of Calcium Oxalate and Uric Acid in Solutions of Urea.

GRACE MEDES.

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The high solubility of calcium oxalate and uric acid in urine has frequently been ascribed to the action of the colloids of urine.[1,2] Urea also is known to increase the solubility of a number of compounds. As the unsaturated amide of carbonic acid it combines with acids to

[1] Joly, J. S., Stone and Calcarous Diseases of the Urinary Organs, St. Louis, 1929.

[2] Pauli and Semac, *Biochem. Z.*, 1909, **17**, 235.

form salt-like compounds, many of which are highly soluble. Urea also forms addition compounds with salts, probably through the residual valence of its oxygen. The experiments recorded below are to ascertain how far urea may be responsible for the high solubility of calcium oxalate, uric acid and sodium urate in urine.

Crystalline calcium oxalate ($CaC_2O_4.H_2O$) was prepared by slowly running 0.250 N $CaCl_2$ and 0.250 N $(NH_4)_2C_2O_4$ simultaneously from burets into boiling water. Crystals formed immediately. The mother liquor was removed by suction, the crystals washed repeatedly with water, and dried.

For the solubility experiments, the calcium oxalate and urea were weighed into 100 cc. volumetric flasks and water added to volume with adjustment of temperature. The solutions were thoroughly mixed, transferred to Florence flasks and shaken over night in a mechanical shaker. The calcium oxalate was determined by a modification of the method of Fiske and Adams.[3] The solutions were filtered through a fine ash-free filter paper and 25 cc. of the filtrates transferred to platinum crucibles, dried on a hot plate and heated in a flame to dull redness for 15 minutes. The ash was dissolved in an excess of 0.02 N HCl and titrated back with 0.02 N NaOH from a calibrated micro-buret using methyl red as an indicator.

TABLE I.

Solubility of calcium oxalate in aqueous solutions of urea (100 mg. of calcium oxalate, $CaC_2O_4.H_2O$ + urea and made up to 100 cc. with CO_2-free water). Temp. 22.6°. The solubility of calcium oxalate is expressed in mg. per 100 cc. of solution.

Urea (gm.)	—	0.06	0.15	0.80	3.00	16.00	50.00
Solubility $CaC_2O_4.H_2O$	0.63	0.66	0.70	0.82	1.28	2.80	8.02

For the uric acid solubility experiments uric acid was purified according to the method of Folin.[4] The experiments were set up as follows:

The desired amounts of uric acid and urea were weighed into volumetric flasks and water added to volume. The solutions were mixed, transferred to Florence flasks and shaken in a mechanical shaker for 1 hour. At the end of this time, the flasks were removed and the solutions allowed to stand for 1 hour for the undissolved uric acid to settle. By this time all the solutions were usually clear except those containing about 10% urea which remained opalescent. Samples of each were transferred to centrifuge tubes and centri-

[3] Fiske, C. H., and Adams, E. T., *Am. Chem. Soc.*, 1931, **53**, 2498.

[4] Folin, O., *J. Biol. Chem.*, 1922, **54**, 153.

fuged until clear, precautions being taken to prevent evaporation. The amounts of uric acid in solution in the various samples were determined by Folin's[5] method for the determination of uric acid in blood.

TABLE II.

Solubility of uric acid in solutions of urea. Grams of urea + 80 mg. uric acid made up to 100 cc. with CO$_2$-free water (with adjustment temperature). The solubility of uric acid is expressed in mg. per 100 cc. of solution. Temp., 26.2°.

Urea	Solubility uric acid	Urea	Solubility uric acid	Urea	Solubility uric acid
gm.	mg.	gm.	mg.	gm.	mg.
—	4.39	6.00	9.21	16.00	8.59
0.16	4.78	8.00	9.73	18.00	7.80
0.40	5.33	10.00	11.52	20.00	7.68
2.00	6.44	12.00	10.67	—	—
4.00	6.83	14.00	9.30	—	—

Sodium urate was prepared by adding sodium acetate to a saturated solution of lithium urate, the precipitate was washed repeatedly in water, filtered off and dried. 200 mg. were weighed into each of two 100 cc. volumetric flasks, 3.00 gm. urea added to one, and each made up to volume at 34°. The 2 solutions were subsequently treated as in the previous experiment and the uric acid in solution determined by the same method. Temperature, 34°.

Solubility of sodium urate in water (mg. per 100 cc. sol.) 142.

Solubility of sodium urate in 3% urea (mg. per 100 cc. sol.) 180.

In the experiments above, urea increases the solubility of calcium oxalate, uric acid and sodium urate. The solubility of the oxalate rises continuously, there being no indication of formation of molecular compounds. The solubility of uric acid increases with increasing concentrations of urea until 10% urea is reached after which there is a slight decrease in solubility—a solubility curve which may be explained as the resultant of 2 opposing forces (a) the peptizing action of urea by which it tends to increase the solubility of uric acid, and (b) some reaction between urea and water by which water is made less available as a solvent. The latter effect would predominate in high concentrations of urea.

The peptizing action of urea may therefore be considered an important factor in maintaining these compounds in solution in urine. When 3% urea is present, 1500 cc. of solution would contain 19.2 mg. calcium oxalate, an amount approximating the average daily output of a normal individual.

1500 cc. of a solution containing 3% urea would dissolve about

[5] Folin, O., *J. Biol. Chem.*, 1930, **86**, 179.

100 mg. of uric acid, while the normal daily output lies at 0.25 gm. or above. Urea, therefore, could not maintain this amount of the free acid in solution. 1500 cc. of 3% urea could dissolve 2.1 gm. of sodium urate, an amount well above the daily output of a normal individual. Ammonium urate has about one-half the solubility of sodium urate, and therefore on a concentrated urine with high uric acid content the limit of saturation would be surpassed. Uric acid is present wholly in the form of the free acid only at a pH of 5 or less and is present wholly as the urate only at a pH of about 8. Hence in most normal urines mixtures of free acid and salt occur.

Conclusions. The peptizing action of urea may be an important factor in increasing the solubility of these sparingly soluble compounds which are normally present in urine at about their saturation limit.

6455

A Natural Infection of the Sharp-tailed Grouse and the Ruffed Grouse by Pasteurella tularensis.

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The widespread destruction of rabbits and grouse in Minnesota during 1925 and 1926 led to a consideration of tularemia in epizootic proportions as a cause of the periodic decimation of wild rabbits and possibly of grouse. Green and Wade[1] reported that ruffed grouse were susceptible to experimental infection with *Pasteurella tularensis*. Parker and Spencer[2] had previously considered tularemia as a possible disease of grouse and had produced a fatal infection in a blue grouse. Tularemia as a natural disease of birds was definitely established by Green and Wade,[3] when they isolated *Pasteurella tularensis* from a quail dying in the wild. Parker,

[1] Green, R. G., and Wade, E. M., PROC. SOC. EXP. BIOL. AND MED., 1928, **15**, 515.

[2] Parker, R. R., and Spencer, R. R., *Sixth Biennial Report of the Montana State Board of Entomology*, 1925-1926, 30.

[3] Green, R. G., and Wade, E. M., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 626.

Philip, and Davis[4] recovered *Pasteurella tularensis* from sage hens, found dead or killed, on an area in which these birds were known to be dying.

In the present paper is reported the isolation of *Pasteurella tularensis* from a sharp-tailed grouse and from a ruffed grouse in Minnesota. In the course of the wild-life disease investigation, routine examinations and study were carried out on game birds collected in various areas of Minnesota during the fall of 1932. Most of the specimens were collected by game wardens, through the cooperation of the State Department of Conservation.

Tularemia in the Sharp-tailed Grouse *(Pedioecetes phasianellus)*. A sharp-tailed grouse, No. B-10419, collected by shooting on September 27, 1932, in Pine County, apparently well when killed, was received at the laboratory on September 28, and 46 ticks of the genus *Hemophysalis* were collected, principally from the bag in which the carcass was shipped. Necropsy of the grouse revealed no abnormal pathology.

First transmission generation. A guinea pig injected with the 46 ground ticks remained well. A guinea pig injected with a suspension of the liver died on the 12th day. This pig showed infiltration and adenopathy in the right inguinal region at the point of inoculation. There were several large abscesses on the liver. The spleen was enlarged but bore no visible lesions. Cultures from the liver and spleen inoculated on glucose-cystine blood agar yielded no growth.

Second transmission generation. Two guinea pigs were inoculated with ground liver and spleen from the preceding guinea pig. One of these was found dead on the 8th day, with local adenopathy and infiltration, and with the necrotic areas typical of tularemia on the liver and spleen. The spleen was used for further transmission. The second guinea pig, which died on the 13th day, showed the usual lesions of tularemia, except for the absence of necrotic areas on the spleen. Cultures on glucose-cystine blood agar remained sterile.

Third transmission generation. Two guinea pigs inoculated by skin scarification died on the 5th and 6th days, respectively, with lesions typical of tularemia. *Pasteurella tularensis* was isolated in pure culture from both the liver and the spleen of the guinea pig dying on the 5th day.

Fourth transmission generation. The infection was transferred

[4] Parker, R. R., Philip, Cornelius B., and Davis, Gordon E., *Public Health Reports*, 1932, **47**, 479.

by scarification with spleen into 2 guinea pigs, which died on the 5th and 7th days, respectively. *Pasteurella tularensis* was isolated from the liver and spleen of both animals. This strain of *Pasteurella tularensis* has been preserved as Minnesota No. 30.

Tularemia in the Ruffed Grouse (*Bonasa umbellus togata*). A ruffed grouse, No. B-10477, was collected, by shooting, in St. Louis County, on October 2. It was reported that six rabbits were seen but that there were few grouse in the vicinity. The carcass was received at the laboratory on October 4. Twelve ticks were obtained from this grouse after arrival at the laboratory.

First transmission generation. A guinea pig injected with 12 ticks, emulsified in saline, remained well. Muscle tissue only[5] was injected from this grouse, as the carcass was somewhat decomposed. The guinea pig injected with the muscle tissue died on the 23rd day, with slight adenopathy and infiltration in the right inguinal region at the point of injection. The spleen was greatly enlarged, and both the liver and the spleen were studded with small necrotic areas.

Second transmission generation. A guinea pig injected subcutaneously with a suspension of liver and spleen died on the 3rd day. Cultures from the liver and spleen yielded *Escherichia coli*.

Third transmission generation. Two guinea pigs and one rabbit were scarified with a suspension of liver and spleen. One guinea pig died on the 5th day, with lesions typical of tularemia. Cultures, however, yielded staphylococcus. The second guinea pig and the rabbit died on the 6th day, both with lesions typical of tularemia. Cultures from the liver and spleen of these animals yielded *Pasteur-*

TABLE I.
Agglutination Tests.

Serum (R.G.G.) preserved in 50% glycerol	10	20	40	80	160	320	640	1280	2560	5120
Minn. No. 30 (Origin, sharp-tailed grouse)										
4 hr. incubation	neg.	neg.	neg.	neg.	2+	3+	4+	4+	4+	4+
12 hr. refrigeration	"	"	"	1+	3+	4+	4+	4+	4+	4+
Minn. No. 32 (Origin, ruffed grouse)										
4 hr. incubation	neg.	neg.	1+	3+	4+	4+	4+	4+	4+	4+
12 hr. refrigeration	"	"	3+	4+	4+	4+	4+	4+	4+	4+
U.S.P.H. No. 38										
4 hr. incubation	4+	4+	4+	4+	4+	4+	4+	4+	4+	3+
12 hr. refrigeration	4+	4+	4+	4+	4+	4+	4+	4+	4+	3+

[5] Green, R. G., and Wade, E. M., PROC. SOC. EXP. BIOL. AND MED., 1929, **17**, 214.

ella tularensis. This strain of *Pasteurella tularensis* has been preserved as Minnesota No. 31.

Identification of the organism was established by the typical lesions of the disease produced by its growth only on a medium containing cystine, by morphological characters, and by the following agglutination tests, which showed a prozone for the organisms isolated from both birds.

The recognition of natural infections of tularemia in quail, sage hens, sharp-tailed grouse, and ruffed grouse, indicates that this disease is widely distributed among species of game bird.

6456

Absorption of Strychnine Sulphate from Strangulated Segments of Bowel.*

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Death, following strangulation of the intestine in man and the experimental animal, is usually attributed to a resulting toxemia. There has been considerable controversy as to the nature of the toxic material, and its route of absorption. To throw light on possible avenues of absorption, a product of known toxicity was introduced into the lumen of strangulated segments of dogs' intestines. Strychnine sulphate was chosen for the toxic substance, as it produced unmistakable clinical symptoms when present in relatively minute doses.

Controls. 50 mg. doses of strychnine sulphate were introduced into the lumen of the normal small intestine of 3 dogs. Similar doses were placed free in the peritoneal cavities of 2 other dogs. All 5 developed definite signs of irritability, followed by convulsions in from 3 to 8 minutes, and subsequent death.

Experiments. 50 to 150 mg. doses of strychnine sulphate were then introduced into the lumen of strangulated segments of bowel. Four types of strangulation were employed. In each type the lumen of the normal bowel, proximal and distal to the strangulated segment, was occluded by tying binding tape firmly about the bowel wall. A small window was first made in the mesentery between the

* This work was supported in part by Grant 224 allowed by the Committee on Scientific Research of the American Medical Association.

vascular arcades adjacent to the bowel wall. The anastomosing vessels running parallel to the bowel wall were then doubly ligated and cut between ligatures, the tape passed about the serosa of the bowel wall and tied. In some experiments the loop chosen for strangulation was isolated by completely severing it from the rest of the bowel, thus making it a closed loop. These experiments are designated in the tables as closed loops (C.L.). In this manner, the segment of bowel selected was completely isolated except for its own vascular bed. The blood supply was then partially or completely obstructed by one of 4 methods. In Group I, all of the arteries to the loop were doubly ligated and severed between ligatures. The veins and lymphatics were left intact in order not to prevent the possibility of venous or lymphatic absorption. In Group II, the veins were doubly ligated and cut between ligatures. The arteries were left intact. The lymphatics, in most instances, were undoubtedly obstructed with the veins, as the mesentery was frequently ligated en masse after freeing the artery from its mesenteric sheath. In Group III, arteries, veins, and lymphatics were all obstructed as the mesenteric pedicle was doubly ligated and cut between ligatures. In Group IV, an encircling ligature of binding tape was passed about the mesenteric pedicle and tied with varying degrees of tension, to obtain variations in the degree of venous and arterial occlusion in order to simulate the conditions that obtain clinically.

In Group I, ten experiments in which the arteries alone were tied, none of the signs of strychnine poisoning, irritability, or convulsions developed during the life of the animals. The average length of life for the group was 16.7 hours. At autopsy, these animals had several hundred cc. of a bloody serous fluid in the peritoneal cavity. The strangulated loops were dark red in color and the lumen was only slightly to moderately filled with a bloody fluid. The wall was about of normal thickness. In no instance was there any evidence of gross perforation. Samples (10-20 cc.) of the peritoneal fluid were injected intraperitoneally into 4 guinea pigs and smaller samples, 3-5 cc., were injected into the dorsal lymph sac of 3 frogs. The pigs did not develop convulsions. The 3 frogs developed delayed convulsions. Convulsions came on in from 12 to 45 minutes.

In Group II, nine experiments in which the veins and lymphatics were ligated, 3 of the 9 animals developed convulsions. The average length of life was 8 hours. At autopsy, the peritoneal cavity contained serous fluid with a bloody tinge. The strangulated loops were dark red in color and their lumen markedly distended with fluid. The loops were extremely heavy in contrast to the undis-

tended loops in Group I. In the 3 cases in which convulsions developed prior to death, bloody fluid from the strangulated loop was found in the lumen of the adjacent normal bowel. This apparently resulted from the extreme distension of the lumen of the loops. This filling of the lumen of the strangulated loop and its subsequent distension was seen in all strangulations of this type and is due, undoubtedly, to seepage of blood through the mucosa of the strangulated loop in consequence of the complete venous obstruction in the face of fully patent arteries which are constantly delivering blood into the wall of the gut. Samples of peritoneal fluid introduced into frogs, as in Group I, were positive for strychnine in 2 instances. In 2 other experiments, there was no evidence of strychnine in the peritoneal cavity after as long a survival period as 20 hours. In 2 experiments, a catheter was tied in the peritoneal cavity and aspirations of the peritoneal fluid were made every 30 to 45 minutes. Three cubic centimeter samples of the fluid were injected immediately upon aspiration into the dorsal lymph sac of 6 frogs. In the first experiment, the first frog developed signs of irritability in 30 minutes and convulsions in 1 hour. The last 5 frogs developed no signs of irritability and none had convulsions. However, the dog had convulsions at the time of last aspiration. In the second experiment, none of the frogs developed irritability or convulsions, neither did the dog have convulsions.

In Group III, ten experiments in which the mesenteric pedicle was completely ligated and severed, 4 animals developed signs of irritability or convulsions during life. The average length of life for the 8 animals that died in this group (2 were killed) was 16 hours. The autopsy findings were almost identical to those in Group I in which the arteries alone had been severed. All of the loops were necrotic, but none showed gross perforations. In 8 instances the frog test for strychnine in the peritoneal cavity at the time of death was negative and in 2 it was positive. In 3 experiments in this group a catheter was tied in the peritoneal cavity and samples of peritoneal fluid were aspirated at hourly intervals for 5 hours and at time of the animals' death; 3-5 cc. of each sample were injected immediately upon aspiration into the dorsal lymph sac of a frog. None of the frogs showed either irritability or convulsions after injection with samples removed during the life of the animals. Two frogs developed convulsions following injection with post-mortem peritoneal fluid. In Group IV, three experiments in which an encircling ligature was placed about the mesenteric pedicle, all of the animals developed convulsions during life. The

average length of life was 8 hours. At autopsy, the findings were quite similar to those in Group II, in which the veins were ligated, the arteries being left intact. Inability to completely arrest absorption through the mesenteric pedicle by an encirclement ligature undoubtedly accounts for the absorption of strychnine in these animals. Frog tests of the peritoneal fluid were negative for presence of strychnine. One animal had a loop with a gross perforation at time of death. Five cc. of this fluid killed a rabbit in 3 minutes when injected intraperitoneally. The rabbit developed marked convulsions within the first minute after injection. A sample of peritoneal fluid taken from this same animal at operation 7 hours previously and 8 hours following the onset of the strangulation was negative for strychnine.

Conclusions. 1. Strychnine sulphate is quickly absorbed from the lumen of the normal bowel and from the peritoneal cavity. 2. Interference with the blood supply of a segment of bowel prevents or

TABLE I.
Arteries Ligated. 50 mg. Given. No Convulsions.

Length	Survival Time	Frog Test
inches	hr.	
6 C.L.	20	+ 45′
30 ”	5	+ 12′
36 ”	14	+ 15′
24 —	7	
30 C.L.	17	Pigs (—)
18 ”	28	” (—)
24 —	21	
36 —	22	
40 —	18	
40 —	15	
	Av. 16.7	

C.L., closed loop. —, regular method (exclusion of segment of gut with binding tape).

TABLE II.
Veins Ligated.

Length	Quant.	Survival Time	Convulsion	Frog Test
inches	mg.	hr.		
40 —	50	6	No	
36 —	”	4	”	Perit. fluid +30′, Loop +5′
36 —	”	10	Yes (8 hr.)	” ” ” ” ”
30 —	”	7	” (2 hr.)	
40 —	”	6½	No	
36 —	”	7½	”	
36 —	”	6	”	
36 —	150	4	Yes	1st Spec. +, Others (—)
36 C.L.	”	20	No	Frog (—), Dog (—)
		Av. 8 hr.		

—, regular method (exclusion of segment of gut with binding tape), C.L., closed loop.

TABLE III.
Complete Division of Mesentery. 50 mg. Given.

Length	Type	Survival Time	Convulsions	Frog Tests
inches		hr.		
18	C.L	30	No	Postmort. spec. +
24	R.	13	"	" " —
12	C.L.	16	"	" " —
36	R.	18	"	5 antemort. spec. —
36	"	20	"	5 " " —
24	"	13	Yes—12 hr.	Postmort. spec. +
24	"	15	" 14 "	" " —
24	C.L.	9	" 6 "	4 antemort. spec. —
30	"	8 (killed)	" 7 "	Postmort. spec. —
15	"	7 (killed)	No	" " —

C.L., closed loop. R, regular method (exclusion of segment of gut with binding tape).

TABLE IV.
Encirclement Ligature.

Length	Quant.	Survival Time	Convulsion	Frog Test
inches	mg.	hr.		
24	150	4½	Yes	(—)
36	"	7	"	(—)
40	"	8	No	
40	(Gross perforation)	15	Yes	Rabbit (+)
		Av. 8½		

greatly delays the absorption of strychnine from the lumen. 3. Complete arrest of blood flow to (arterial occlusion) or from the bowel (venous occlusion) probably precludes absorption through the mesenteric vessels. 4. Evidence of transperitoneal absorption was demonstrated in some of these experiments but only through gut wall whose nutrition was seriously damaged. In the absence of gross perforation or rupture of the gut wall, transperitoneal absorption is slight even in the presence of a badly damaged wall.

Missouri Section.

Washington University Medical School, November 9, 1932.

6457

Effect of Phrenicectomy upon the Efficiency of Cough and upon Elimination of Lipiodol from Lungs.

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The removal of a phrenic nerve to paralyze one-half of the diaphragm is now extensively performed in certain cases of pulmonary tuberculosis and in other conditions. Although the literature contains many statements to the effect that cough and expectoration are facilitated by phrenicectomy we have been unable to find any recorded experimental work dealing with such a result. Our experiments show that on the contrary the operation actually interferes with cough and diminishes the elimination from the lung of lipiodol injected into it.

The efficiency of the cough mechanism was measured by the elimination of lipiodol from the lungs before and after phrenicectomy. The rate of disappearance of the oil was determined by means of fluoroscopy and serial roentgen-ray films. Experiments were performed upon 10 dogs. In 2 dogs the time required for the complete disappearance of the oil from the lung was determined. In these 2 dogs a unilateral phrenicectomy was performed after all the oil had disappeared. After they had recovered from their operations the rate of disappearance of lipiodol was again determined and the effect of phrenicectomy upon this rate noted. In 8 dogs unilateral phrenicectomy was performed first and then equal amounts of lipiodol were injected into the right and left lower lobes.

Following the phrenicectomy it was noted under the fluoroscope that some of the oil injected into the lung on the side of the normal diaphragm was coughed up either during the injection or immediately thereafter. Fluoroscopic observations indicated that the

cough mechanism was interfered with on the side of the paralyzed diaphragm.

The difference in the rate of elimination of lipiodol from the lung fields of those dogs which were subjected to preliminary phrenicectomy and injection of lipiodol simultaneously into both lower lobes is recorded in Table I. The tabulated results indicate that either right or left phrenicectomy resulted in a slower disappearance of the oil on the side of the paralyzed diaphragm.

TABLE I.
Effect of Phrenicectomy Upon Elimination of Lipiodol from the Lung.
(Preliminary Phrenicectomy Followed by Injection of Oil)

Amt. Lipiodol Injected Each Lung	Side of Phrenicectomy	Lung from which Oil Disappeared Completely First	No. Days	Retardation of Elimination, Result of Phrenicectomy
cc.				
3	Left	Right	49	Marked
5	Right	Left	63	Definite
2½	Left	Right	88	Slight
3	Right			Not appreciable (63 days)
6	Right	Left	36	Marked
3	Left	Right	36	"
4	Right	Left	14	Definite
3	Left	Right	14	"

In 2 other dogs the oil was injected on the same side both before and after phrenicectomy. In both instances there was retardation of the elimination of oil subsequently to phrenicectomy as compared with that following the original injection.

6458

Superior Vena Cava Obstruction.

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The effects of superior vena cava obstruction vary according to the degree and location of the obstruction and the suddenness of its development. To determine the tolerance of animals to this condition, to measure the effect on venous pressure and to trace the paths of collateral circulation, experimental obstruction of the superior vena cava was produced in dogs.

The vein was exposed through an intercostal incision and com-

pletely occluded by dividing between ligatures. The usual aseptic technique was followed and the operations were performed under intratracheal ether anesthesia administered with positive pressure.

Obstructions were of 2 types, (1) above the junction of the azygos vein, and (2) including the azygos vein. Two attempts were also made to obstruct the superior vena cava below the junction of the azygos vein but both dogs died within a few minutes. Obstruction above the level of the azygos vein was produced in 7 dogs. One died within 24 hours and 3 died within 12 to 14 days as a result of a propagating thrombus or an empyema. Three recovered. Of the latter, one was sacrificed for study after 30 days. Another was later subjected to azygos vein obstruction and the third is alive after more than 5 months. One dog survived the immediate effects of a 2-stage obstruction of the superior vena cava and azygos vein but died at the end of 21 days from an infected bilateral pleural effusion.

The most striking general results of superior vena cava obstruction were cyanosis of the tongue and oral mucosa and injection of the conjunctivae. Somnolence, listlessness and slow deep respirations were also noted. The dogs with obstruction just above the right atrium, dying within a few minutes, had extreme cyanosis of the upper part of the body. The surviving animals recovered from their cyanosis within 48 hours and developed dilated veins over the thorax and abdomen.

Venous pressure readings were obtained by inserting a needle into the exposed external jugular vein and measuring the height of a column of blood in an attached vertical glass tube. The original pressures varied between 4 cm. and 13 cm. of blood. These pressures were increased 100% (4 readings) immediately after the obstruction. They were 65% higher (3 readings) during the first 9 days and were not significantly increased (3 readings) 14 to 29 days after the obstruction.

The venous pressure after obstruction including the azygos vein was obtained in one dog. The original reading was 6 cm. The pressure 7 days after the second stage procedure (occlusion of the azygos vein) was 16 cm. of blood, an increase of 167%. As this dog died, further readings were not obtained.

Collateral circulation was studied by injecting a barium mixture into the veins and taking roentgen-ray films, and by dissection. In one dog methylene blue was added to the injection mixture to facilitate dissection. These injections revealed a system of anastomosing veins which included the following: (1) Superficial veins,

chiefly the thoraco-epigastric, superficial epigastric and a superficial plexus of veins of the thorax and abdomen. (2) Deep veins, including the internal mammary, intercostals, azygos, hemiazygos, accessory hemiazygos, anterior and posterior mediastinal, pericardiaphrenic, phrenic, superior and inferior epigastric, lumbar, and deep anastomosing veins to the back muscles.

When the obstruction was above the junction with the azygos vein, the azygos system was a very important path of return flow to the heart but the abdominal collaterals were not well developed.

In the dog with obstruction of the superior vena cava including the azygos vein the flow of blood in the azygos system was evidently reversed and the superficial and deep abdominal collaterals carrying blood below to the femoral or iliac veins or to the inferior vena cava were much more prominent.

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A Study of Pannus Formation in the Cornea of Rabbits.*

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In the transmission of experimental trachoma to animals, perhaps the most significant and refractory difficulty is the complete lack of corneal involvement. Except in man where infection followed accidental or intentional transfer of trachomatous material. pannus formation has not accompanied folliculosis experimentally induced in a variety of animals. Since the appearance of cicatricial changes and pannus comprise the essential diagnostic differentiation between trachoma and folliculosis, the necessity is obvious for studying the conditions under which vascularization of the cornea is stimulated. While it is not yet clear that the data thus far obtained may be correlated eventually with pannus formation in trachoma, it is of interest that pannus may be evoked by various stimuli. From this point of view the experiments undertaken are here reported.

The experiments have been done on rabbits;† and because the results of a former study indicated that pannus may be a mani-

* This work was done under a grant from the Commonwealth Fund of New York.

† A total of 138 rabbits was used, about half for the experiments described

festation of bacterial hypersensitiveness, it was decided to study vascularization of the cornea as an allergic response. Rabbits have been sensitized to different bacteria: *Staph. aureus*, *Bact. granulosis*, Pneumococcus, diphtheroids and indifferent streptococci. Various methods for inducing hypersensitiveness of the cornea to bacteria were studied, but the most successful one was scarification of the cornea with one scratch reaching from below the sclero-corneal margin to the pupil, and then instilling into the conjunctival sac 2 drops of a young, actively growing culture. This treatment was repeated at weekly intervals. *S. aureus* proved to be the most effective sensitizing antigen, while *B. granulosis* was one of the least.

The experiments with *S. aureus* indicate that following the first 3 or 4 instillations, no visible reaction takes place. From the third to the fifth inoculations, the cornea undergoes a very definite reaction to the bacteria characterized by vascularization and increasing cloudiness which may be due to a keratitis. The vascularization begins in the form of delicate blood vessels springing from the injected conjunctival vessels. With repeated inoculations the corneal vessels form small fine loops which show anastomoses and later descend over the cornea not as loops but as a curtain of single vessels which terminate in crow-foot or brush-like processes. Eventually vessels appear from all directions, reaching centripetally from the entire circumference of the cornea. As long as the instillations are continued it is possible to keep the pannus and clouding present, so that in several animals by continuing inoculations for 22 weeks, pannus was maintained for months. The cornea undergoes clearing as the inoculations are discontinued and in a few weeks the eye regains grossly its normal appearance.

It has been determined also that vascularization of the cornea may be stimulated by injection of toxic materials, by mechanical injury, and by infection. Consequently the observation was occasionally made while studying the corneal changes to repeated infection that pannus may be stimulated by the first inoculation of bacteria. In these instances the pannus usually begins to appear within a week and does not require repeated treatments.

To determine whether the reactions described are due entirely or in part to repeated infection, hypersensitiveness, or some other factor, further experiments have been conducted in which the cornea has been sensitized to purified crystalline egg albumen. Rabbits

on bacterial sensitization, and 30 more in studying sensitization to albumen. Thirty-five were used in preliminary experiments leading to the ones reported at the present time.

were injected directly into the cornea or inoculated by combined scarification and instillation of egg albumen once a week. It has been possible to reproduce the corneal changes described above. The rabbits receiving intracorneal injections begin to show corneal changes within 3-4 injections, and eventually develop a very severe reactivity to egg albumen. In the rabbits receiving egg albumen by instillation and scarification, the reaction develops more slowly and never so severely. Rabbits receiving intracorneal injections of sterile physiological salt solution on the other hand show no increasing reactivity to successive injections.

While it is possible, therefore, to stimulate pannus formation in the cornea of rabbits under various conditions, it is clearly shown that vascularization of the cornea may accompany direct sensitization to live bacteria, or native proteins such as egg albumen.

6460

Effect of Acid Extract of Anterior Pituitary on Heart Rate, and Nervous Irritability of Guinea Pigs.*

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It has been observed that the injection of acid extract of cattle anterior pituitary produces in guinea pigs a condition comparable to that seen in Graves' disease in human beings. Loeb[1] has found a marked hypertrophy of the thyroid gland with diminution in the amount of colloid and a marked increase in the number of mitoses in the epithelial cells. Under the action of this substance the animals lose weight, and Siebert[2] has shown that there is an increase in their metabolic rate. Other effects corresponding to conditions found in Graves' disease have been established in subsequent investigations carried out in this laboratory. Inasmuch as tachycardia and nervous irritability are very prominent symptoms of Graves' disease in man it was of interest to study the effect of this extract of anterior pituitary on the heart rate and the reflex irritability of guinea pigs.

* These investigations were in part carried out with the aid of a grant for research in science made to Washington University by the Rockefeller Foundation.

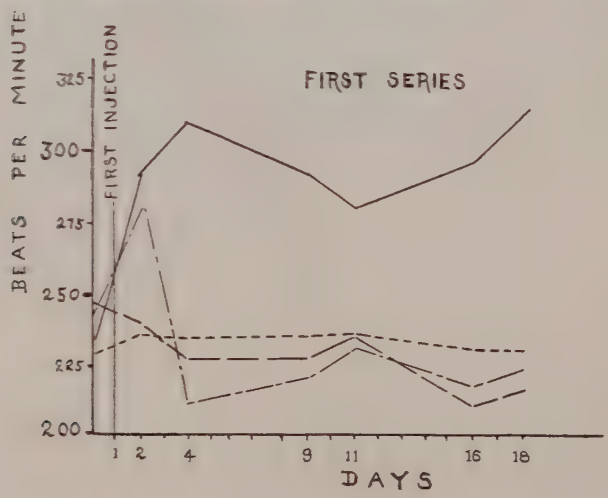
[1] Loeb, Leo, and Bassett, R. B., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 860.

[2] Siebert, W. J., and Smith, R., *Am. J. Physiol.*, 1930, **95**, 396.

Cursory observations of Loeb seemed to indicate that such an effect might possibly exist.

Twelve young male guinea pigs, approximately 200 gm. each, were chosen and normal records were obtained under a standard procedure using the electrocardiograph (lead one) to record the cardiac pulsations. Daily injections of 1 cc. of the acid extract were given to each of 6 guinea pigs, 3 of which had been thyroidectomized. The remaining 6 guinea pigs, 3 of which had been thyroidectomized, were used as controls, and were not injected. Records of the heart rate of all the animals were obtained on the 2nd, 4th, 9th, 11th, 16th, and 18th days. The heart rate of every animal in the group of injected non-thyroidectomized animals was faster on each occasion, after the second day of the injections, than that of any of the animals in the other groups. The rate of the thyroidectomized non-injected (control) animals dropped considerably below normal. In the case of the injected thyroidectomized animals the rate also went below normal, but not as low as that of the thyroidectomized control group, except at the time of the last observation after the death of several animals had rendered the averages used in plotting the graph unreliable. (First series in Fig. 1.) Since some of these animals died and because the number originally used was small, the experiment was repeated to check the accuracy of the results.

In the second series, 17 animals were used and 3 determinations of their heart rates were made before the injections of acid extract



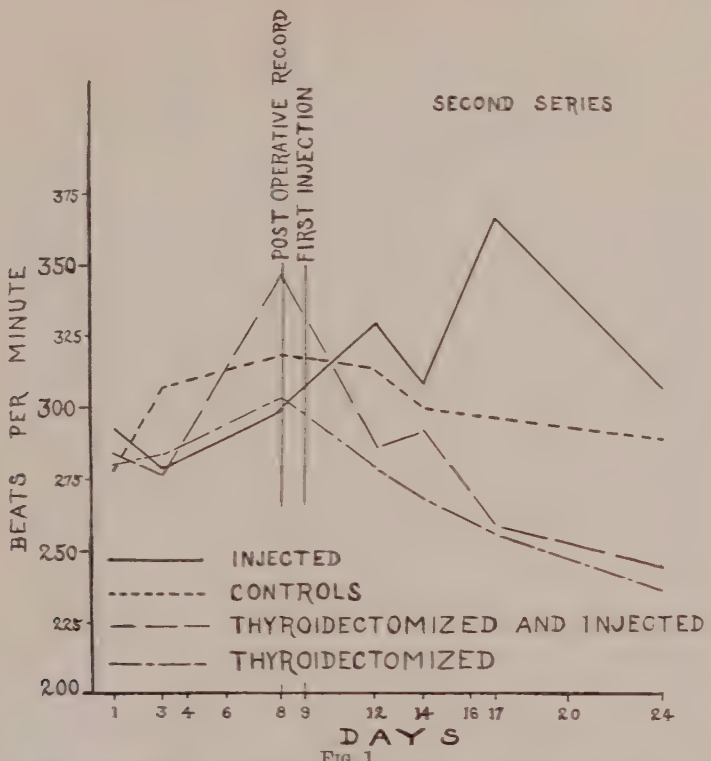


FIG. 1.

Graphic representation of the heart rates of the 4 groups of animals during the course of the experiments. The curves in each case are plotted from the average number of beats per minute of all the animals in a particular group.

were started. The animals were then divided into 4 groups and determinations and observations were made on the 3rd, 5th, 8th, and 15th days after the injections were begun. The results were similar to those obtained in the first series. The thyroidectomized animals, whether injected or not, showed heart rates slower than those of the normal animals, and in each instance the rate was below that observed in the same animal before the experimental procedures were instituted. An increase of 70 or more beats per minute was observed in the case of the normal animals injected with acid extract. The maximum increase in beats per minute in the individual animals was 71, 86, 96 and 105.

In the first series, the rate rose until the fourth day after the injections were started, it then dropped until the eleventh day, after

which it rose steadily. In the second series the initial rise was not as rapid, and the maximum rate was observed on the eighth day, after which it declined. We may conclude that acid extract of the anterior pituitary of cattle is effective in increasing the heart rate of the guinea pig when the thyroid gland is intact. This substance increases the heart rate, in the main, indirectly by acting through the thyroid gland. A slight increase in the heart rate was observed in the injected thyroidectomized animals which may have been due to small remnants of thyroid tissue. However, this increase was, on the whole, much less marked than in the non-thyroidectomized injected animals.

In our first series, the respirations were counted throughout the experiment and the results seemed to indicate that the rate of respiration varies directly as the heart rate. Our observations on the second series were less complete and less convincing. The respirations were counted for a period of one minute by 2 different observers, several counts being made of each animal, but the counts varied so much, even in the case of the same animal, perhaps due to the necessity for handling them, that we regard the results on respiration merely as suggestive.

An attempt was made to determine whether any difference in excitability or in degree of response to a given stimulus was noticeable between the animals of the several groups. The response of an injected and a non-injected animal to the same stimulus was always compared, and in many instances, when graded stimuli were used, the injected animal reacted to a smaller stimulus than the normal animal. We made use of an ear reflex that one of us had studied in connection with an infectious otitis media and interna in the rat.[3] When a sudden noise occurs near a rat or guinea pig the animal moves its ears backward and then forward. This motion usually occurs once, but an unusually loud noise, or one repeated several times in rapid succession, may elicit several such movements. A dull noise was produced by striking the table upon which the 2 animals to be tested were resting. Graded stimuli could be produced by gradually increasing the force with which the table was struck. In comparing the reactions of an injected and a normal animal a stimulus could be found to which the injected animal responded by moving the ears, but which was without effect upon the normal animal. In many cases by the time the stimulus was increased to the point of eliciting only the ear motion in the normal

[3] McCordock, H. A., and Congdon, C. C., PROC. SOC. EXP. BIOL. AND MED., 1924, **22**, 150.

animal, it caused the injected animal to jump and shudder several times before quieting down.

Since many of the animals, even the controls, showed individual variations, an attempt was made in the second series to pair the animals as equally as possible. We selected as a pair 2 animals that showed nearly equal heart rates and as nearly as possible the same degree of response to noise stimuli. One of these was used as a control animal and its partner as an experimental subject. The response of these 2 animals to an identical stimulus was always compared at the same time.

With the elimination of the individual variations that this pairing accomplished, the increase in response to stimuli on the part of injected animals over the controls was more striking. In the first series one of the 3 injected animals always showed an increased response to stimuli regardless of the control animal with which it was compared. However, the increase in irritability of the other 2 injected animals was less marked, and the control guinea pigs showed such individual variations in response to stimuli, that either the control or the injected animal could be made to respond first to a given stimulus, by selecting a certain type of control. In the second series this variability in response was successfully eliminated by pairing the animals in the manner stated above. Observations were made every other day and after the fifth injection the difference in the strength of reactions of the control and injected animals became quite definite in each case. The injected animal of each pair always responded to a weaker stimulus than did its control partner. These results apply to animals with intact thyroid glands. On the other hand, if thyroidectomized control and injected guinea pig were compared no appreciable difference was observed in the reactions of these two kinds of animals.

Because of the individual variations in the nervous excitability of guinea pigs as manifested by their response to noise stimuli it is suggested that in experiments of this nature more uniform results can be obtained by pairing the animals on the basis of observations made before the experimental procedures are started.

The facts established add further data of interest concerning the similarity between the changes caused by the injection of acid extract of the anterior pituitary gland and those observed in Graves' disease in man.

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Dorsal Root Fibers Which Contribute to the Tract of Lissauer.

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Recent physiological evidence shows that the small, thinly myelinated fibers of peripheral nerves are concerned with the conduction of pain (Heinbecker, Bishop and O'Leary[1]). Evidence for the participation of fiber elements in the tract of Lissauer of the spinal cord in the transmission of pain seems adequate (Ranson and Billingsley[2]). It is necessary, therefore, to determine how large is the number of small, thinly myelinated fibers which enter the tract of Lissauer from the dorsal roots.

To examine this tract, the seventh lumbar segment of the spinal cord of the cat with attached dorsal roots was isolated and divided into lateral halves. One half was fixed in ammoniated alcohol for the pyridine silver technique, the other in 2% osmic acid by immersion. The osmic acid cross-sections revealed that Lissauer's tract contains closely packed, small, thinly myelinated fibers. The silver pyridine preparations showed from 1½ to 2 times more fine axons than could be accounted for by the number of fibers apparent in the osmic acid sections. The additional axons were those of non-myelinated fibers.

Evidence secured by Ranson[3] through degeneration of the dorsal roots indicates that in the lumbosacral cord (cat) the tract has medial and lateral divisions; the lateral is primarily endogenous to the cord, the medial contains fibers which have entered from the dorsal roots.[4] Accordingly, in 7 cats, under ether anesthesia, the dorsal roots (levels L. 5 through S. 3) were ligated and cut (3) or crushed (4) between the spinal ganglia and the cord. The cats were killed after 20 to 100 days and the material prepared by the silver pyridine, 2% osmic acid and Marchi methods. The results show that a majority of the small, thinly myelinated fibers in the medial half of the tract of Lissauer degenerate (cord levels L. 6 and L. 7). Likewise a similar proportion of the non-myelinated fibers are

[1] Heinbecker, P., Bishop, G. H., and O'Leary, J., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 925.

[2] Ranson, S. W., and Billingsley, P. R., *Am. J. Physiol.*, 1916, **40**, 371.

[3] Ranson, S. W., *J. Comp. Neurol.*, 1914, **24**, 531.

[4] Ranson, S. W., *J. Comp. Neurol.*, 1913, **23**, 259.

eliminated. Both fine, myelinated and non-myelinated (endogenous) fibers remain.

In osmic acid preparations secured after 20 to 25 days there was no evidence of intact myelinated fibers in the central stumps of such divided dorsal roots. In silver pyridine preparations, however, occasional intact fibers were observed. These had the appearance of regenerating fibers but to remove doubt completely, 3 spinal ganglia were removed in each of 8 cats. After an adequate time was allowed for degeneration, the material was prepared by the pyridine silver method and sections cut through the zone of entry of the degenerated dorsal roots. In more than 20 such roots, evidence of the occurrence of intact fibers, either myelinated or non-myelinated, was lacking; only 3 or 4 intact non-myelinated fibers being observed in the whole series. Thus, both the small, thinly myelinated fibers and non-myelinated fibers of the dorsal roots have their cells of origin in the dorsal root ganglia.

Our results show that a larger number of small, thinly myelinated fibers are contributed to the tract of Lissauer by the dorsal roots than would be inferred from the conclusions of Ranson.[3] The dorsal roots undoubtedly contribute more non-myelinated than small, thinly myelinated fibers, but the question of whether pain is mediated by one or the other group is not to be decided by an excess of numbers. For example, the large, thickly myelinated fibers, known to be responsible for the conduction of tactile impulses, are no more numerous in the dorsal roots than the small, thinly myelinated ones. The finding that a significant number of small, thinly myelinated fibers in the tract of Lissauer are derived from the dorsal roots removes one difficulty in inferring that pain is mediated by this group.

6462

Allocation of Function to Specific Fiber Types in Peripheral Nerves.

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In a recent communication (Heinbecker, Bishop and O'Leary[1]) the fibers in a skin nerve associated with the sensations of touch and of pricking pain were identified. Knowing that the fibers responsible for such pain are included in a well-defined group of small myelinated ones, and that those for touch fall in a different group of larger fibers, we have two points of reference for locating other functions. The differential susceptibility of nerve fibers to novocaine permits a further assignment of various functions mediated by nerves, to known fiber groups. Such fiber groups can be recognized by their potentials when stimulated, and in novocaine anesthesia the differential blocking of these potentials can be correlated with the differential disappearance of sensations.

Observations were made on 3 surgical patients to determine the order of disappearance and reappearance of various sensations and of skin temperature changes during low spinal anesthesia produced by dissolving novocaine crystals in spinal fluid. The changes noted were first a very early increase in skin temperature, after 2 to 3 minutes a loss of sensitivity to heat and cold, and then to cutaneous and pressure pains. The order of disappearance of these sensations was definitely distinct but the time interval between their disappearance was brief. After a distinctly wider gap there occurred a loss of motor function and after another short interval a loss of joint sense, sensation of pressure and touch. In recovery the order of sensibility return was reversed with the gaps between the return of different sensations much wider.

In vitro experiments to determine the effect of novocaine on the action potential of the cat's saphenous nerve definitely showed that the first potential to disappear was that derived from unmyelinated fibers. Then the potential derived from the small somatic myelinated fibers and finally the potential derived from the large thickly myelinated fibers of this nerve were eliminated.

A correlation of the experimental data now permits an allocation of specific functions to specific fibers in a skin nerve. The first

[1] Bishop, George H., Heinbecker, Peter, and O'Leary, James, PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 928.

New York Meeting.

New York Academy of Medicine, December 21, 1932.

6463

Etiology of an Uncomplicated Coryza in the Domestic Fowl.

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The domestic fowl is subject to an infectious coryza, generally known as contagious catarrh, which under laboratory conditions remains localized in the nasal and orbital passages. In this respect it is unlike the coryza of laryngotracheitis, fowl pox, and fowl cholera from which there is commonly an extension of the etiological agent to other loci with the production of characteristic lesions.

Contagious catarrh was recently studied in Holland by deBlieck,[1] who isolated an organism resembling the *B. influenzae* of man from exudate streaked on an aerobic blood agar plate. Pure cultures of the bacterium when injected intranasally in normal fowl regularly produced a nasal discharge.

In the present investigation an uncomplicated coryza was initiated and subsequently maintained in a flock of disease-free birds by the intranasal injection, through the palatine cleft, of exudate originally obtained from naturally infected fowl. A nasal discharge, the only consistent symptom, generally appeared after an incubation period of 24–48 hours and continued, on the average, for 11 days.

The etiological agent of the coryza was not established by the injection either of bacteria isolated from aerobic plates or of exudate filtered through Berkefeld V and N candles. The bacteria were the usual mucous membrane inhabitants, among them several strains of a hemophilic bacillus similar to *B. influenzae*.

It was subsequently found, however, that the fluid from blood agar cultures of exudate which had been filtered through certain

[1] deBlieck, L., *Vet. J.*, 1932, **88**, 9.

Berkefeld V candles contained a small, non-motile, Gram-negative bacillus apparently in a pure state. The organism grew sparsely in fluid blood at the base of slanted agar but failed to colonize on the slant or on the surface of aerobic blood agar plates. Colonization was later initiated by sealing the plates with modeling clay.

A typical coryza was produced in normal birds, 35 in all, by injecting the bacillus into the palatine cleft. The same organism was recovered from the nasal exudate induced in these birds, at first by filtration and later by the use of sealed plates. The duration of the period of nasal discharge, averaging 5 days, was shorter than that of the coryza produced by exudate. There was also an indication that the bacillary coryza was less communicable by direct contact. Thus, of 5 normal birds in contact with 5 which had received an injection of the bacillus, 2 developed coryza, whereas all of the 5 in contact with a similar number injected with exudate developed coryza.

It was found that recovery from the coryza of bacillary origin was commonly followed by a state of resistance to reinfection. The time limits of the period of resistance were not determined. Cross protection tests were subsequently carried out to ascertain whether there was any fundamental difference between the coryzas produced by exudate and culture, respectively. Four birds which had recovered from the bacillary coryza were resistant to an infective amount of exudate and a similar number which had recovered from the coryza produced by exudate were resistant to an infective amount of culture. It is believed that the demonstration of reciprocal protection establishes the etiological relation of the bacillus to the coryza.

Cultural studies on the organism are not complete, but its failure to colonize on aerobic blood agar plates together with an inability to multiply in media containing sufficient accessory material of plant origin to support growth of *B. influenzae* appears to exclude it from the group of true hemophilic bacteria.

6464

Identity of the Viruses Causing "Mad Itch" and Pseudorabies.

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In experiments already reported[1] it was shown that while "mad itch" virus regularly caused rapidly fatal infections in cattle, cats, rabbits, mice and guinea-pigs, it produced in swine, when injected subcutaneously, only a temporary illness. Serum from swine that had recovered from this infection neutralized both the "mad itch" and pseudorabies viruses* as was shown by injection of mixtures of viruses and serum into susceptible guinea pigs. These facts indicated that the 2 viruses were closely related if not identical, but their relationship could not be determined conclusively at that time because serum of animals immune to pseudorabies was not available.

Schmiedhoffer[2] has reported that pseudorabies virus was without effect when injected into swine although von Ratz[3] observed the disease occurring naturally in wild swine. In our hands the pseudorabies virus has produced a febrile disease of a few days' duration. After recovery the serum from such swine neutralizes the virus.

With serum available from swine that have recovered from pseudorabies and with serum from swine recovered from "mad itch", cross neutralization tests to determine the relationship of the 2 viruses have been made and are given in Table I.

The data show that serum from swine recovered from either "mad itch" or pseudorabies neutralizes both viruses. The conclusion to be drawn, therefore, is that the inciting agents of "mad itch" and pseudorabies are immunologically identical. The differences in the experimental disease produced by the 2 viruses[1] must then be considered only as variations induced by 2 strains of the same virus. In recognition of the identity of "mad itch" as occurring in this country and pseudorabies as occurring in Hungary and other European countries, in any future publications the "mad itch" virus will be designated as Pseudorabies Virus (Iowa Strain).

[1] Shope, R. E., *J. Exp. Med.*, 1931, **54**, 233.

* The "mad itch" virus was obtained from a cow in Iowa.[1] The pseudorabies virus was kindly furnished by Professor Alădar Aujeszky, of the Hungarian Veterinary School in Budapest.

[2] Schmiedhoffer, J., *Z. Infektionskrankh. Haustiere*, 1910, **8**, 383.

[3] von Ratz, S., *Z. Infektionskrankh. Haustiere*, 1914, **15**, 99.

TABLE I.

Cross Neutralization of the Pseudorabies and "Mad Itch" Viruses.

Subcutaneous injection of 1.5 cc. containing 1 cc. of virus (10% suspension of virus-containing rabbit brain)	0.5 cc. serum† swine	Guinea Pig No.	Result
Pseudorabies	673—normal	506	Died—71 hr.—control
"	" "	507	" 80 " "
"	810 "	514	" 68 " "
"	" "	517	" 66 " "
"	" "	543	" 63 " "
"	1235—pseudorabies convalescent	500	No illness
"	" " "	501	" "
"	" " "	502	" "
"	" " "	503	" "
"	1185 " "	494	" "
"	" " "	495	" "
"	772—"mad itch" convalescent	522	" "
"	" " " "	524	" "
"	" " " "	542	" "
"	" " " "	546	" "
"Mad itch"	673—normal	504	Died—73 hr.—control
" "	" "	505	" 90 " "
" "	810 "	515	" 105 " "
" "	" "	516	" 72 " "
" "	" "	547	" 77 " "
" "	1235—pseudorabies convalescent	496	No illness
" "	" " "	497	" "
" "	" " "	498	" "
" "	" " "	499	" "
" "	1185 " "	526	" "
" "	" " "	527	" "
" "	772—"mad itch" convalescent	518	" "
" "	" " " "	521	" "
" "	" " " "	544	" "
" "	" " " "	545	" "

† The serum and virus were mixed and stored in the refrigerator over night (17 hours) prior to inoculation into the test guinea pigs.

6465

Herpes Antiviral Substances; Distribution in Various Age Groups and Apparent Absence in Individuals Susceptible to Poliomyelitis.

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That the virus of herpes simplex of human origin is capable of producing neurotropic changes in the rabbit and certain other rodents was shown by Doerr[1] and others. Since the work of Zinsser

[1] Doerr, R., *Z. f. Haut- u. Geschl.*, 1924, **18**, 27.

and Tang,[2] Andrewes and Carmichael[3] it has been known that the blood serum of many individuals contains an antibody which may cause *in vitro* neutralization of this virus so that the mixture becomes non-pathogenic for these animals. Examinations of human sera for herpes antibody have for the most part been made to trace some connection between epidemic encephalitis and herpetic infection, symptoms and pathology of the latter when produced in rodents showing some similarity to the former disease in man. The results of such tests have been negative, convalescent encephalitis patients having approximately the same antibody incidence as is found in "normal" individuals.

Brain[4] showed fairly definitely that an attack of herpes in man always gives rise to the production of more or less permanent blood-born antibodies though these antibodies give no apparent protection against future attacks. Because the white mouse is susceptible to herpes virus when introduced intracranially (Andervont[5]) it has been possible to study conveniently the action of neutralizing serums with a fair degree of accuracy. The availability of a large series of human sera from known age groups suggested the desirability of studying herpes virus neutralizing capacity at various ages.

To determine the ability of a serum to neutralize, equal volumes of centrifugalized 5% emulsion of virus-containing mouse brain and the serum under investigation were mixed and allowed to remain at room temperature for about an hour (time unimportant) before intracranially inoculating two 18-20 gm. white mice under light ether anesthesia. In order that the virus material be of as uniform potency as possible throughout the tests brains were used only from mice which had died during the fourth or fifth day after inoculation. The volume of inoculum was invariably 0.05 cc. to each mouse and duplicate inoculations were made throughout. Control mice received 0.05 cc. of a 2.5% and a 0.5% suspension, the former representing the same amount of virus received by the test mice while the latter dilution served to give an index of the potency of the virus through the delay in symptoms caused by this further dilution. As all mice receiving the 0.5% virus died, the test mice were injected with a minimum of 5 killing doses of virus, either neutralized or not depending upon the serum in the mixture. However, accurate

[2] Zinsser and Tang, *J. Immunol.*, 1929, **17**, 343.

[3] Andrewes and Carmichael, *Lancet*, 1930, **1**, 857.

[4] Brain, *Brit. J. Exp. Path.*, 1932, **13**, 166.

[5] Andervont, *J. Inf. Dis.*, 1931, **49**, 507.

titrations of the virus at frequent intervals showed that the mice were in reality receiving upward of 50 killing doses.

In general, the mice which received the serum-virus mixtures either died about the fifth day, or remained well. Occasionally the symptoms were retarded in development but this was an exception, and if the illness did not become apparent until after the tenth day the result was considered positive for neutralization. Such mice usually lapsed into a comatose state in which they lingered for as long as 2 weeks. Another form of discrepancy occasionally met was that one mouse of a pair died promptly while the other failed to die but showed symptoms with ultimate improvement. In this event the serum was usually retested and in general the result was such that we may assume either that the surviving mouse had unusual individual resistance to the virus or that the inoculation had not been properly made.

The results have been tabulated according to the ages of the individual donors in groups of a 5-year interval. Above the age of 45 the sera were few and were grouped together.

TABLE I.

Age Group	No. Sera Tested	% Neutralizing
0-5	28	14
5-10	26	38
10-15	11	40
15-20	19	90
20-25	28	90
25-30	14	71
30-35	22	68
35-40	16	75
40-45	10	65
45 and over	13	54

These results are quite in harmony with those of Zinsser and Tang,[2] who reported neutralization in 25% of sera from children below the age of 7 years, 43% in "children in general" and what would appear to be 64% neutralization in adults if we may strike an average on his statistics dealing with "normal" adult and convalescent encephalitic adult sera.

The sera upon which our observations were made came from several geographical sources, eastern United States, the West Indies, and China. There is nothing to indicate that geography plays any appreciable part in the reactions.

One would, of course, assume that the antibody develops in the individual as a response to herpetic infection. In view of the ubiquity of herpes simplex it would be extremely difficult to exclude

the rôle of the virus. Friedberger *et al.*[6] similarly finding in human sera "antibodies" capable of serological reactions with sheep and rabbit erythrocytes came to the conclusion that these substances are non-specific in the serum and express processes which arise physiologically during the maturation of the individual. Their figures indicate that such antibodies are not found in the blood of a child at birth but that they appear as the age of the individual increases so that 90% of the sera from persons above 10 years show a positive reaction despite the fact that no parenteral contact with sheep or rabbit antigens would normally be supposed. Coca's work[7] on the diffusibility of native proteins perhaps weakens this argument for the natural acquisition of antibodies as a feature of maturation. That a similar curve has been observed through the age groups with respect to the antitoxins for diphtheria and scarlet fever need scarcely be mentioned except that the available figures show that the same curve obtains in geographical locations from which these diseases are absent.

Of the sera available for examination from the standpoint of herpes antibody as reported above, 27 were specimens drawn from children who proved to have been susceptible to poliomyelitis, all of them victims of the 1931 epidemic. The bleedings were in most cases "initial" ones, *i. e.*, they were made upon diagnosis of poliomyelitis for the purpose of neutralization tests with the virus of poliomyelitis. The results of the tests with herpes virus showed them to be individually and collectively devoid of any neutralizing effect. A comparison of the results with the incidence for herpes virus neutralization in the same normal age groups gives:

TABLE II.

Age Group	No. sera, non-polio. patients	% neutralizing herpes virus	No. sera, polio. patients	% neutralizing herpes virus
0-5	16	17	12	0
5-10	18	50	8	0
10-15	9	55	2	0
Age?	—	—	5	0

The neutralization tests upon the poliomyelitis sera were repeated twice using a minimum of 6 test mice upon each serum. This absence of herpes antibody among persons subject to poliomyelitis suggested the possibility that either the absence of herpes virucidal bodies was indicative of the absence of poliomyelitis antibody, or vice versa, or, it might show that a single "panimmune body" was

[6] Friedberger, *et al.*, *Z. f. Imm.*, 1929, **64**, 294.

[7] Coca, *J. Imm.*, 1930, **19**, 405.

lacking from the serum which if present would accomplish the neutralization of either or both viruses.

In previous experiments the blood serum from several animal species, viz., hen, rabbit, mouse, guinea pig, sheep, horse and monkey was tested for a herpes neutralizing factor. A *Macaccus rhesus,* the serum from which caused a 5-day delay in the appearance of herpetic symptoms in test mice, was the only animal in this instance which gave any evidence of possessing virucidal antibodies. This monkey was a convalescent poliomyelitis animal which had recovered from a mild attack of experimentally induced poliomyelitis 6 months previous to the time of the bleeding tested. The result led to the testing of 4 additional monkeys; one normal animal gave no trace of neutralization. The second, a convalescent which had been further "reinforced" by the inoculation of additional virus after recovery which gave complete neutralization. The third, a convalescent from the 1931 virus, which also gave complete neutralization. The fourth monkey had been given poliomyelitis virus intradermally but at no time had it shown paralytic symptoms. The serum did not neutralize the herpes virus.

The normal monkey control in the group just described was then given 5% herpes virus intradermally over a period of 3 weeks, 10 cc. of suspension being used in all. The serum now caused a retardation in the herpetic symptoms in test mice of from 4 to 5 days. Difficulty has been experienced in producing a high titre serum not only in the non-susceptible monkey but also in the highly susceptible rabbit and mouse in absence of infection. Recovery from infection in the latter animals, however, produces a serum of considerable potency. The most potent anti-herpes monkey serum came from an animal which had not received herpes virus but which had acquired poliomyelitis antibodies through an experimental poliomyelitis infection.

Conclusions. An antiviral substance which has the ability to neutralize the virus of herpes simplex has been found to be present in the blood early in the life of a few individuals. As age advances, though not necessarily because of maturation, more individuals acquire the antiviral property. A peak is reached in early adult life, after which apparently a decline sets in.

A group of sera from children whom we may assume carried no antiviral substances for poliomyelitic infection were shown likewise to be devoid of antiviral substances toward herpetic infection. Finally, herpes virus neutralizing properties have been discovered in the blood of monkeys which have reacted to poliomyelitis virus.

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Variation of H. Influenzae During Acute Respiratory Infection in the Chimpanzee.

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In a previous communication[1] the striking resemblance of the bacterial flora of the upper respiratory tract of the chimpanzee to that of human beings has been described. To what extent these animals possess a similar flora in their native environment, or to what extent the organisms present represent new acquisitions after landing in the United States is not known.

The chimpanzee is highly susceptible to the common cold, whether naturally communicated by contact with infected human beings or artificially induced by bacterium-free filtrates of naso-pharyngeal washings from subjects suffering from acute colds. One of the striking phenomena of these chimpanzee colds is the great increase both in numbers and in area of distribution during the early stages of the cold, whether spontaneous or experimental, of the common pathogens of the upper respiratory tract such as *H. influenzae, pneumococcus* and *S. hemolyticus.*

In particular, we have noticed from the beginning of our study of the common cold in apes, during periods of infection, a change in the number and character of the colonies of *H. influenzae* on rabbit's blood agar plates. During the healthy period cultures from the naso-pharynx and nose give few colonies of *H. influenzae* from the naso-pharynx and usually none from the nose. The colonies themselves are small and opaque. During the period of infection numerous colonies of *H. influenzae* are obtained both from the naso-pharynx and the nose. Furthermore, the colonies themselves are of an obviously different character, very large, translucent and moist.

A more careful study of this phenomenon has revealed facts of great interest and importance. In April, 1932, an outbreak of spontaneous colds occurred among our chimpanzees. Eight animals became infected and from all of them during the first days of the cold *H. influenzae* was isolated. Four of the strains were carefully studied bacteriologically and serologically by Dr. Margaret Pittman

[1] Dochez, A. R., Shibley, G. S., and Mills, K. C., *J. Exp. Med.*, 1930, **52**, 701.

of the Hospital of the Rockefeller Institute. By the methods[2] which she has devised for the classification of *H. influenzae* all 4 strains were demonstrated by the use of the Levinthal medium to be the S. form of *H. influenzae*. Serological study further revealed that 3 of the strains belonged to immunologic Group A and one to Group B. From 5 to 8 weeks later the same 4 animals were examined for the presence of *H. influenzae*. From one no *H. influenzae* was recovered. From 3, *H. influenzae* was obtained and the 3 strains isolated were proven bacteriologically to be the R. form of the organism. No S. forms were present on the plates made at this time. One animal that developed bronchopneumonia as a sequel of its cold carried the S. form for 5 weeks.

In June, 1932, another spontaneous outbreak of colds occurred among the animals. From 7 of the 8 animals having colds at this time *H. influenzae* was isolated. All of the 7 strains obtained represented the S. form of *H. influenzae*. Serological examination of the strains isolated showed 4 of the animals to harbor the Group A variety, 2 Group B, and one both Group A and B. Twelve colonies picked from a single plate were all of the S. form. Practically all of the colonies obtained at this time by frequent plating of the upper respiratory flora were examples of the S. form of *H. influenzae*. Only very rarely was the R. form of colony visible on the plate. The serologic variety of *H. influenzae* obtained from the 4 animals studied during the June outbreak was in each instance the same as that obtained in the previous attack of cold studied. Of 2 animals kept in the same cage one harbored *H. influenzae* Group A, and the other *H. influenzae* Group B.

To throw further light on this phenomenon, 2 chimpanzees were inoculated with fresh bacteria-free filtrates of the naso-pharyngeal washings from human beings with acute colds. Chimpanzee No. 1 was carefully studied for the presence and type of *H. influenzae* during a foreperiod of several days. *H. influenzae* was obtained by plate culture and numerous colonies examined were found to be of the R. form. The animal was then inoculated intranasally with the fresh filtrate from a human cold. Cultures from the naso-pharynx within 48 hours from the time of inoculation revealed the presence of numerous S. forms of *H. influenzae*. By the third day the S. form had entirely disappeared and only R. forms were obtained. This animal developed no clinical symptoms of the common cold.

Chimpanzee No. 2 was studied during a similar foreperiod under

[2] Pittman, Margaret, *J. Exp. Med.*, 1931, **53**, 471.

careful quarantine and again only R. forms of *H. influenzae* were isolated from the naso-pharynx. The animal was then inoculated with filtrate from a common cold and the naso-pharyngeal flora studied bacteriologically. Again, within 48 hours large numbers of colonies of *H. influenzae* appeared on the plates and all colonies examined were of the S. form. In this instance the S. form of *H. influenzae* was obtainable on cultures during a period of 6 days. The animal showed symptoms of a mild cold. Serological study of the type of *H. influenzae* present following experimental inoculation showed this type to be the same as that observed in each animal during the previous periods of natural infection, in each instance Group A.

These observations indicate that under the influence of infection of chimpanzees with the virus of the common cold, a transformation takes place from the serologically non-type specific R. form of *H. influenzae* to the serologically specific S. form. During health periods there is reversion to the R. form. The presence of such small numbers of S. forms as to be undemonstrable during healthy periods and an increase to the point of demonstrability during periods of infection cannot be ruled out, but seems improbable. Introduction of S. forms from the outside cannot be an explanation of the phenomena observed because of the presence of more than one serological type of *H. influenzae* in the group of animals during the epidemic period, and because of the recurrence in each animal of the identical serologic type of organism throughout a series of infections. Furthermore, in one instance of the controlled experimental study of the phenomenon such a careful quarantine was maintained that introduction of organisms from the outside environment was rendered extremely unlikely. These observations confirm our previous studies which indicate that one of the most important effects of the virus of the common cold is to incite activity on the part of potentially pathogenic micro-organisms present in the naso-pharynx at the time of infection. Whether this action is directly operative upon the micro-organism influenced or is an indirect effect of tissue reaction cannot be stated.

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Removal of Intravenously Injected Antigen by Circulating Precipitin.

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Numerous investigators have reported that a specifically immunized animal eliminates intravenously injected antigen more rapidly than does a normal animal.[1, 2, 3] Evidence has been presented to show that the precipitin antibody is of importance in the disposition of the foreign material.[4] Virtually nothing has been established, however, as to whether the circulating or a fixed-tissue precipitin plays the major rôle in this elimination. The attempts to determine the relative importance of the circulating precipitin and the fixed-tissue precipitin in the removal of the injected antigen have been hindered by the lack of suitable methods for quantitatively estimating this antibody in an antiserum. Recently, we[5] proposed a procedure, which we called the "neutralization method," for quantitatively titrating the precipitin against crystalline egg albumin. This method seems to overcome the difficulty in estimating precipitin and appears advantageous for investigating the relative importance of the circulating and fixed-tissue antibody in eliminating antigen.

We have been able by the neutralization method to determine quantitatively the effect on the circulating precipitin of the intravenous injection of antigen into an immunized rabbit. The amount of antibody, expressed in milligrams of nitrogen per cc. of the serum, present just before and one hour after injecting a known amount, in milligrams of nitrogen, of crystalline egg albumin has been found experimentally. The total circulating precipitin of the rabbit has been calculated by multiplying the amount of precipitin determined per cc. of serum by the number of cc. in the animal's plasma volume. The plasma volume has been considered, from determinations we have recently offered,[6] to approximate 3.4%

[1] Weill-Halle, B., and Lemaire, B., *C. R. Soc. Biol.*, 1906, **61**, 114.

[2] Smith, G. H., and Cook, M. W., *J. Immunol.*, 1917, **2**, 421.

[3] Hempl, H., *J. Immunol.*, 1917, **2**, 141.

[4] Glenny, A. T., and Hopkins, B. E., *J. Hyg.*, 1922, **21**, 142; 1923-24, **22**, 12, 208.

[5] Culbertson, J. T., *J. Immunol.*, in press.

[6] Culbertson, J. T., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 102.

of the body weight of the rabbit. Data, suggesting the essential importance of the circulating precipitin in removing the intravenously injected antigen, are presented in Table I for 8 rabbits of a longer series studied in this regard.

TABLE I.

The effect of the intravenous injection of antigen on circulating precipitin.

Plasma volume (cc.)	Antigen injected (mg.N.)	Antibody plasma vol. before test injection (mg.N)	Antibody plasma vol. 1 hr. after test inject. (mg.N)	Antibody neutralized by test inject. (mg.N.) Exper. (Diff. columns 3 and 4)	Theoretic. (Antibody the antigen would neutralize "*in vitro*")
64	1.51	49.64	28.22	21.42	19.63
66	3.02	41.14	6.83	34.31	39.26
72	1.51	60.86	32.64	28.22	19.63
73	1.51	50.02	33.02	17.00	19.63
81	3.02	33.66	None*	33.66*	39.26
89	0.76	46.92	37.40	9.52	9.88
91	1.51	59.50	35.70	23.80	19.63
95	4.53	54.74	None*	54.74*	58.89

* Antigen detected in the blood taken 1 hour after the injection of antigen.

The experimental data show that the mean difference between the amount of precipitable antibody in the plasma of the rabbit prior to the test injection and that after the injection is approximately equal to the amount of antibody which would be precipitated by the given amount of antigen in the test tube. The ratio of union of antigen with antibody is apparently the same both for the union *in vitro* and for the combination in the circulation of the immune animal. After an amount of antigen greater than that calculated to neutralize the precipitin of the plasma of the rabbit is introduced into the circulation, free antigen but no precipitin is present in the blood. After an amount of antigen less than that necessary to neutralize the precipitin of the plasma volume is introduced, no antigen is present in the blood and the precipitable antibody of the plasma is reduced in proportion to the quantity of antigen injected. This result indicates that the antibody immediately available for the disposition of intravenously injected antigen is contained entirely in the blood plasma.

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A Syndrome Produced in the Dog by Inclusion of Oxidized Fat in the Diet.

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The present investigation was undertaken to determine whether the chemical condition of the fat of a ration influenced the ability of an experimental animal to maintain health. McAmis, Anderson and Mendel[1] and Burr and Burr[2] have described a disease in rats due to the absence of fat in an otherwise complete diet. The syndrome is characterized by failure in growth, changes in the skin, especially of the tail and feet, and kidney degeneration. Burr and Burr[3] later reported that the disease could be prevented or cured by the feeding of linoleic acid. Evans and Lepkovsky[4] confirmed Burr and showed that glycerides of saturated fatty acids do not cure nor prevent the disease, but fatty acids containing more than one double bond have remarkable ability to improve fat-free diets. The conclusion was that certain fatty acids, essential for wellbeing, cannot be synthesized by the rat, although the rat is able to produce body fat on diets deficient in these essential fatty acids. Most of the work on fat feeding has been concerned with neutral fat or pure fatty acid. There have been a few contributions on the effects of fats altered in various ways. Powick[5] and Fridericia[6] showed independently that rancid fat destroyed the vitamin A of a ration when the fat came in contact with the vitamin-containing material.

The investigation to be reported deals with the effect on dogs of fat subjected to oxidative rancidity. A basal diet of purified foodstuffs planned to be adequate in all known dietary essentials was prepared: casein 16%, sucrose 55%, fat 25%, agar 2.5%, salt mixture 1.44%, and cystine .06%. It was very low in vitamins

* This study was aided by a grant from H. D. Pease.

† An abstract of this work was presented before the Food and Nutrition Section of the American Public Health Association in Washington, D. C., October 26, 1932.

1 McAmis, A. J., Anderson, W. E., and Mendel, L. B., *J. Biol. Chem.*, 1929, **82**, 247.

2 Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1929, **82**, 345.

3 Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1930, **86**, 587.

4 Evans, H. M., and Lepkovsky, S., *J. Biol. Chem.*, 1932, **96**, 165.

5 Powick, W. C., *J. Agric. Research*, 1923, **31**, 1017.

6 Fridericia, L. S., *J. Biol. Chem.*, 1924, **62**, 471.

and was supplemented with Harris yeast concentrate and oscodal,‡ a cod liver oil concentrate. The vitamin concentrates fed were considered adequate for normal growth and wellbeing, and to insure complete consumption were given in gelatin capsules 4 hours before the rest of the ration was offered. The lard was Armour's 5-Star Brand. To produce an oxidative rancidity in the fat a stream of oxygen was bubbled through it at a temperature of 60°C. until the undiluted material showed a decided color with the Kreis test, but when diluted 1:10 with purified kerosene no color was produced.

Three control dogs receiving the basal diet with its low nitrogen (0.3 gm. N per kilo of dog) supplemented with yeast concentrate and oscodal remained in good health. Two of these dogs were used for other purposes at the end of a 4 months' control period. One is still in good health after 11 months. Four additional dogs, fed the same ration (supplemented with the vitamin concentrates), except that the neutral fat had been replaced by oxidized fat, became ill; the time of onset of the symptoms was quite variable, but the progress of the disease was strikingly similar in all affected animals. The earliest sign was a loosening and falling out of the hair; in some dogs this took place all over the body, but more frequently the hair over the face was lost first. The hair over the feet and legs was next to go, then that over the abdomen; sometimes that over the back could be pulled out by handfuls. The dogs remained lively and well during this period, sometimes even gaining weight. Within a variable time after the first loss of hair was noted, a rash appeared and spread over the body. About this time the appetite began to decline and the dogs became less lively. The stools were very black and inclined to be hard. The skin lesions became worse, ulcers appeared over the bony protuberances and rapidly became deep, sometimes exposing the bone. There was very little inflammation about them, and they did not seem to be tender. The dogs became weaker and frequently convulsive movements of the extremities were noticed. The constipation gave way to diarrhea, which was usually blood-tinged and frequently frankly bloody. The animals became weaker and finally died. The eyes remained unaffected to the end; the mouth was never involved.

The normal mouth seen in this symptom complex distinguished it from the disease produced by Underhill and Mendel[7] and from that produced by Goldberger.[8] We are led to believe that the presence of

‡ Supplied by H. A. Metz Co.

[7] Underhill, F. P., and Mendel, L. B., *Am. J. Phys.*, 1929, **83**, 589.

[8] Goldberger, J., *U. S. Public Health Reprints*, 1928, **43**, 172.

oxidized lard was in some way responsible for the syndrome of loss of hair, skin lesions, anorexia, emaciation and intestinal hemorrhages observed in our dogs. As our control dogs remained in good condition, we are convinced that this pathological condition is not due to an inadequacy in our basal ration.

Several mechanisms of action by which partially oxidized fats may bring about this syndrome may be mentioned. It might be a direct toxic effect upon the animal, though this seems unlikely. It might produce a greater need for one of the vitamins or call for a different balance between them. It is possible that the action of the oxygen on the fat destroyed some important grouping such as the unsaturated linkage shown by Burr and Burr[3] to be essential for health in the rat. The signs and symptoms presented by the dog do not resemble very closely those described for the rat, but there may be a marked species variation.

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Blood Volume in Normal Chicks and in Chicks with Nutritional Encephalomalacia.

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The cerebral lesions in the disorder of chicks described by Pappenheimer and Goettsch[1] as *nutritional encephalomalacia,* appear unquestionably to result from vascular disturbances. Oedema, hemorrhage and hyaline thrombosis of capillaries are the conspicuous initial features of the lesions and they must be due either to alteration of the vessel walls, or to a quantitative or qualitative change in the blood itself.

In attempting to analyze the problem from this point of view, it seemed of interest to determine first whether the disease producing Diet 108* leads to an alteration in the total volume of blood. This paper presents briefly experiments bearing on this phase of the subject.

Material and Technique. Eighty 2-day-old chicks were placed on Diet 108 and 20 controls of the same hatch on the natural foods Diet

[1] Pappenheimer, A. M., and Goettsch, M., *J. Exp. Med.*, 1931, **53**, 11.

634 of Hogan, Hunter, and Kempster.[2]† Additional control material on Diet 634 was also available. The chicks were of 4 different breeds, White Leghorns, White Wyandottes, Rhode Island Reds and Barred Plymouth Rocks, but it may be stated here that no significant correlation was obtained between breed of chick and blood volume. Neither was any difference found in the susceptibility to this disease, the percentage incidence being approximately the same in all breeds.

The determinations of the plasma and blood volume were carried out by the dye method described by Graff and Clarke,[3] but with certain minor variations. A 1.3% solution of K oxalate was found to be optimal for chicken erythrocytes, giving neither crenation nor hemolysis. A 1% solution of brilliant vital red in 0.9 NaCl solution filtered and sterilized, was used for injection. It was convenient with chicks to inject a much greater volume of dye in proportion to body weight than it was in humans. This permitted the withdrawal of smaller samples as was necessary with young chicks. Our dilution factors were hence approximately 10 times those of Graff and Clarke. The syringe (Tuberculin-Becton-Dickenson & Co.) was calibrated, and a suitable correction made in the calculations.

The technique was as follows: Under ether anesthesia, the heart was punctured through the chest wall, and with the needle in the ventricle, an accurately measured amount of the dye solution injected. Later this was modified by opening the thorax and injecting directly into the heart. After 5 minutes ($\pm 10''$), the chick being kept under light anesthesia, the heart was aspirated, and the blood added to small test tubes containing weighed amounts of 1.3% potassium oxalate. Tube A, containing approximately 0.1 cc. of oxalate, was used for hematocrit determinations. Tubes B and C, containing 2.0 cc. of oxalate, were weighed, centrifuged, and the diluted plasma used for duplicate readings in a Koenig-Martens

[2] Hogan, A. G., Hunter, J. E., and Kempster, H. L., *J. Biol. Chem.*, 1928, **77**, 431.

* Diet 108 has the following composition

	%
Skimmed Milk Powder (Merrel-Soule)	15.0
Casein (Merck's technical)	20.5
Cornstarch	20.0
Lard	21.0
Cod Liver Oil	2.0
Yeast (Fleischmann's bakers, dried)	5.0
Salt Mixture (McCollum 185)	6.5
Paper Pulp (Eastman)	10.0

† Diet 634 of Hogan, Hunter and Kempster:

	%
Whole Wheat	55.6
Whole Milk Powder	8.2
Casein	12.3
Alfalfa Meal	2.5
Butter Fat	4.2
NaCl	0.9
$CaCO_3$	1.3
Cod Liver Oil	3.0
Yeast	12.0

[3] Graff, S., and Clarke, H. T., *Arch. Int. Med.*, 1931, **48**, 808.

spectrophotometer. For further details as to the optical technique, see Graff and Clarke.[3]

Precision of the Methods Used. 1. *Sampling Time.* One of the recognized difficulties in the application of the dye method is to obtain samples at the precise time when mixing has been completed, and before there has been an appreciable loss of dye from the circulating blood. These time relationships have been studied in detail in human blood volume determinations by Graff, d'Esopo, and Tillman.[4] They have shown that mixing is completed within 4 to 7 minutes after injection of the dye. This is followed by gradual disappearance of the dye at the rate of 10 to 20% per hour. The curves obtained in chickens from samples secured by repeated sampling at short intervals resembled those in humans, save that the rate of loss of the dye was very much greater. Thus 3 curves showed losses of 17.5%, 19.5%, and 20% per 10 minutes after mixing. This difference in the rate of dye loss probably has no physiological significance since a much greater proportionate volume of dye was used in the chicks. The rate of loss is probably a function of the concentration.

Although this rapid loss of dye undoubtedly introduces inaccuracy as regards the absolute value of the figures obtained, it seems probable that samples obtained at exactly identical times after injection of the dye will yield comparable values. As has been stated, the samples were taken in precisely 5 minutes ($\pm 10''$) after dye injection.

2. *Divergence of Checks in Duplicate Determinations.* This indicates the errors in the dye method which are the combined result of errors in hematocrit determinations, specific gravity, and spectrophotometric estimations. These individual sources of error may be briefly discussed. From our data of 93 hematocrit determinations, the P. E. is calculated as 0.23. This implies a maximal deviation of about 0.53% in the cell volume values in our series.

3. *Specific Gravity.* In our calculations a specific gravity of 1.060 has been assumed. Actual determinations of heparinized chicken blood gave specific gravity values of 1.046, 1.045, 1.047, and 1.044, an average of approximately 1.045. This shows an error of approximately 1.5% in the specific gravity and a slightly lower error in the final calculation. Thus in one example, recalculation

[4] Graff, S., d'Esopo, D. H., and Tillman, A. J. B., *Arch. Int. Med.*, 1931, **48**, 821.

using the specific gravity figure 1.045 gives 55.4 cc. plasma per kilo, instead of 54.8 cc. This is, therefore, not an important source of error, if we assume that the specific gravity of the blood under the experimental conditions is reasonably constant.

4. The error in the spectrophotometric estimation is the usual one of approximately 2%. In the estimation of duplicate samples from 67 individuals, the P. E. was 2.51%.

5. *Error Due to Varying Weight of Intestinal Contents.* A possible source of error which, if ignored, would lead to differences in the final calculation, is the varying weight of the intestinal contents, which may, as is shown in the following table, amount to 10% of the body weight. The weight of the alimentary contents including those of crop and gizzard, was estimated by weighing the intestinal tract full, and after washing out the contents. The mean average weight expressed in percentage of body weight in 25 chicks on the experimental Diet 108, was 7.4%; in 17 chicks on the control Diet 23, 7.5%.

During the first few days there is considerable uniformity; later the individual variations are wide, but there is no constant difference between the 2 diets.

It would seem desirable in estimating the blood volume, to deduct the weight of the alimentary contents from the body weight. Unfortunately, the contents were not weighed in the earlier determinations of this series. We have, therefore, introduced into these calculations an arbitrary deduction of 7.5% of the body weight, as approximating the weight of the alimentary contents, and recalculated our blood volume estimations with this correction.

Blood and Plasma Volume in Normal and Affected Chicks. The determinations are recorded in Table I and Table II,, which show convincingly that there is no significant difference between the two groups. The data may be summarized as follows:

Diet	108	634
No. Chicks	31	38
Aver. Plasma Vol. (cc./kilo)	56.1	55.0
Aver. Plasma Volume (corrected for wt. of alimentary contents)	62.2	59.7
Aver. Blood Volume (cc./kilo)	83.4	82.9
Aver. Blood Volume (corrected for wt. of alimentary contents)	91.0	95.4

Since the P. E. in the methods is at best 2.5% and possibly somewhat higher, it is obvious that the slight difference between the two groups is not significant.

TABLE I.
Chicks on Diet 634 (Normal Controls Without Symptoms or Lesions).

Age	Breed	Wgt.	C/V%	Uncorrected Plasma cc./kilo	Uncorrected Blood cc./kilo	Wt. of Alimentary Contents	Corrected Plasma cc./kilo	Corrected Blood cc./kilo
1	1	38	33.5	57.0*	81.0			
1	1	33	31.8	56.0*	83.5			
1	1	30	28.6	60.5*	84.5			
1	1	34	32.5	48.0*	77.0			
3	1	55	31.9	55.5	81.0	E. 4.1	59.6	87.2
3	1	50	34.0	45.6	69.0	E. 3.7	49.0	74.5
3	1	45	32.4	55.0	81.5	E. 3.4	59.7	88.5
4	1	54	31.9	40.8	60.0	6.5	48.2	75.0
4	1	51	34.6	56.0	85.5	E. 3.8	65.0	92.5
4	1	38	28.9	51.6	73.0	E. 2.8	56.0	78.6
5	1	56	33.3	55.7	83.5	E. 4.1	60.0	89.9
5	1	48	36.1	50.2	81.0	3.5	54.5	87.0
5	1	53	33.9	52.5	80.0	4.5	57.5	87.0
6	1	61	41.4	52.5	89.6	4.5	56.8	97.0
10	1	70	34.6	49.5	76.0	6.5	55.5	84.6
11	1	75	36.7	54.5	86.0	7.5	60.5	96.0
11	1	93	38.4	49.0	79.5	9.5	54.5	88.0
12	1	78	33.8	57.0	86.0	3.5	62.0	93.6
15	1	105	33.8	56.5	85.0	7.0	60.5	97.0
19	1	93	41.0	63.0	106.0	E. 6.9	66.0	111.0
22	1	188	32.0	52.0	77.0	16.5	57.4	84.5
23	2	139	35.4	54.0	83.5	E. 10.5	58.5	91.5
26	1	204	33.7	45.0	68.0	11.5	47.7	72.0
27	1	195	36.0	48.6	76.0	19.0	54.0	84.5
27	1	96	30.4	72.5	104.0	E. 7.2	78.0	112.0
28	1	185	43.7	43.5	77.2	10.5	46.0	81.7
30	2	245	34.4	55.0	84.0	18.3	59.5	91.0
33	3	244	23.3	59.0	77.0	18.2	64.0	84.0
33	3	290	30.8	51.8	75.0	21.8	56.0	81.0
34	1	203	31.5	53.5	78.5	15.2	58.0	84.5
39	3	331	37.8	57.5	92.5	24.8	62.2	100.0
43	1	275	37.8	55.0	88.4	20.6	59.0	95.0
48	3	357	34.9	52.1	80.6	26.7	56.6	88.0
?	2	426	26.4	55.5	75.5	32.0	60.0	82.0
?	2	357.5	35.1	59.3†	91.2	E. 27.3	68.0	100.5
?	2	218	33.7	84.2‡	126.0	E. 16.4	91.0	137.0
?	2	293	28.7	56.6	71.2	E. 22.0	61.7	86.6
?	2	109	32.0	69.9	100.2	E. 8.2	76.0	111.5

* 1 day chicks, no alimentary contents.
† Sampling time long; . ·. high value.
‡ Sampling time less than 5 minutes.
1 = White Leghorn; 2 = Rhode Island Red; 3 = Barred Plymouth Rock.
E = Estimated at 7.5% of body weight.

Relation of Blood Cells to Plasma in Normal and Affected Chicks. The data are given in Tables I and II. The average cell volume percentage in 50 chicks on control Diet 634, was 33.0% with a σ of 4.65. On the experimental Diet 108, with 43 chicks, the average was 34.0, and a σ of 5.03. If this second group is subdivided into those showing pronounced symptoms and lesions, and those showing no or only slight changes, we find in the former group (28

TABLE II.
Chicks on Diet 108.

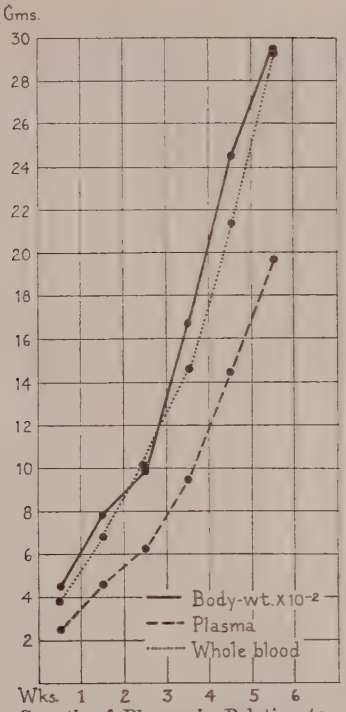
Age	Breed	Wt.	Symptoms	Lesions	C/V%	Uncorrected Plasma Blood		Wt. of Alimentary Contents	Corrected Plasma Blood	
						cc./ kilo	cc./ kilo		cc./ kilo	cc./ kilo
18	1	98	+++	++++	35.1	66.3	102.0	E. 7.4	74.5	115.0
19	1	76	+++	++	31.2	45.0*	65.0	E. 5.7	51.2	75.5
20	4	128	+++	—	34.6	57.8	88.0	E. 9.6	63.5	99.0
20	4	112	±	—	30.9	63.6	91.5	E. 8.4	69.0	99.0
21	1	99	++++	++++	38.0	50.7	81.7	10.5	56.5	91.2
22	1	88	+++	+++	41.0	74.6	126.0	E. 6.6	80.5	111.0
23	2	78	+++	+++	33.0	56.0	83.5	E. 5.8	61.0	91.0
24	2	85	++++	++++	33.6	68.0	93.0	E. 6.3	66.0	99.0
24	4	120	++++	+++	31.2	63.6	93.6	E. 9.0	68.5	99.5
25	2	97	+++	+++	35.6	61.0	94.7	E. 7.3	66.0	105.0
26	1	111	+++	+++	31.7	47.2	69.0	10.0	51.7	76.0
27	1	111	+++	++	34.1	39.2†	58.6	7.0	41.0	62.5
27	3	137	+++	+++	23.8	77.0	101.0	E. 8.3	83.0	108.5
27	4	91	+++	+++	33.2	66.0	99.0	E. 6.8	72.5	109.0
28	1	146	++++	++++	34.2	49.6	75.5	5.0	51.5	78.5
29	1	178	++++	++++	37.4	61.5‡	98.5	11.5	66.5	106.0
29	4	319	+	+	35.4	51.5	79.6	23.9	55.5	86.0
32	3	191	++	—	36.2	61.7§	97.0	14.3	67.0	104.0
32	2	129	+++	+++	24.4	59.8	79.0	9.7	65.0	86.0
35	1	199	+++	++++	39.4	48.2	79.5	19.0	53.5	88.0
35	1	263	+++	+	35.2	43.6	67.5	18.0	46.8	72.2
35	1	207	++++	++++	36.6	52.8	83.2	7.0	54.8	86.5
40	4	214	+++	+++	33.8	48.5	73.5	16.0	52.6	81.0
40	2	155	++	+++	39.0	60.5	99.0	11.6	63.5	104.0
40	2	194	—	+	43.9	48.9	87.2	E. 14.5	52.0	92.5
43	4	285	—	—	23.5	55.5	74.5	21.3	60.0	81.0
47	1	256	—	—	39.6	50.8	84.0	19.2	55.0	91.0
48	2	260	±	+++	52.0	48.0	100.0	19.5	52.0	108.0
49	2	247	+	+++	35.9	51.0	80.0	18.5	55.0	86.5
49	3	270	±	+	29.7	55.0	78.3	20.5	59.7	85.0
50	4	248	—	—	35.6	56.0	87.5	E. 18.6	58.2	90.5

* Moribund.
† Moribund. Heart beat very slow.
‡ High figure may be due to rapid loss of body weight from 192 to 172 gm. Recalc. for body weight of 192 gm.: 57 Pl/k. 91 Bld/k.
§ Stopped breathing immediately after injection. Heart stopped after second puncture.
1 = White Leghorn; 2 = Rhode Island Red; 3 = Barred Plymouth Rock; 4 = White Wyandotte.
E. = Estimated at 7.5% of body weight.

chicks), an average cell volume of 32.8%, with a σ of 5.13; in the latter (15 chicks), 34.6% with a σ of 4.98.

It is apparent from these figures that there is no significant difference in any of the groups; in other words, the disease is not accompanied by alterations in the cell plasma ratio.

Relation of Blood Volume to Age and Weight; Growth of the Blood as a Tissue. As is shown in Table III, our data included determinations on normal chicks from one day to 6 weeks or more



Growth of Plasma in Relation to Growth of Body Weight.

FIG. 1.

in age. There is thus afforded opportunity to study the growth of the plasma and whole blood in relation to the body weight and age during the early growth period. In Fig. 1 is shown (1) the weight of the chicks during the first 6 weeks, the points on the curve representing the average weight of the chicks killed in each 7-day period; (2) the average volume of the plasma, and (3) of the whole blood compiled from the same group of chicks.

The data upon which the graph is constructed are briefly summarized in the following:

TABLE III.

Week	No. Chicks	Aver. Wt. gm.	Plasma cc.	Whole Blood cc.	Wt. of Blood/Body Weight
1	14	46	2.6	3.9	.09
2	4	79	4.6	6.9	.09
3	2	99	6.2	10.3	.11
4	6	168	9.5	14.7	.09
5	4	246	14.5	21.5	.09
6	8	296	19.8	29.6	.10

It is obvious that within the age limits of the experiment, there is an extraordinarily close correspondence between the growth of the blood and plasma, and that of the body weight as a whole. This is borne out by the constancy in the relation between blood weight (calculated by multiplying volume by specific gravity factor 1.06), and the body weight.

Conclusions. 1. The disease described as nutritional encephalomalacia of chicks is not associated with significant alterations in cell plasma ratio, plasma or blood volume. 2. During the early growth period of the chick, the growth of the plasma and blood follow closely the growth of body weight. The blood and plasma volume per kilo, aside from individual variations, remain constant throughout the early growth period.

6470

The Central Nervous System in Relation to the Digestive Functions.

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In this communication a comparison is made between certain secretory, motor and vascular phenomena produced by pilocarpine administered into the cerebral ventricles of monkeys, and the corresponding responses to the drug when given subcutaneously.

A series of 15 green monkeys *(Lasiopyga callithricus)* has been used. On 6 of them, fractional gastric analyses have been made following the administration by stomach tube of 100 cc. of a farina test-meal. Several experiments have been conducted on each animal to demonstrate the action of the drug pilocarpine (hydrochloride), when administered at various times in the course of digestion of the test-meal. The drug has been given in varying doses subcutaneously and by the intraventricular route.

The intraventricular injection of pilocarpine, in doses of 5-10 mg. per kilo of body weight, invariably caused a sudden and complete cessation of the gastric secretion of free HCl, at whatever stage in the digestion the drug was given. The samples obtained after pilocarpine injection consistently failed to show any free HCl acid.

The total acidity curve usually paralleled the 'free acid' curve but at a higher level. After intraventricular injection of pilocar-

pine, the total acid generally fell *pari passu* with the 'free' acid. This fact, together with the lessened volume of recoverable gastric contents, indicates that the phenomenon is not merely one of neutralization by mucus or regurgitated bile. In one monkey with a low type of acid curve the level of total acidity merely ceased to rise. The 'free' acid in this case fell to zero.

If a subcutaneous injection of histamine (1-2 mg. per kilo) was given in the achlorhydric phase following intraventricular pilocarpine administration, the "acid tide"—both 'free' and 'combined'—was rapidly restored and reached a high level.

Subcutaneous injection of pilocarpine did not produce these striking effects upon the gastric secretion. Doses of 20-30 mg. per kilo (*i. e.,* 4 or 5 times as large as the intraventricular dose) failed to prevent the "acid tide" and did not produce achlorhydria, although there was some lowering of the curves. Smaller doses were without effect.

The increase in motility of the stomach was evidenced by the short emptying time after pilocarpine administration, in doses of 5-10 mg. per kilo, whether it was given by the subcutaneous or intraventricular route. In the latter case the difference was quantitative, both in its general and specific effects, and manifested itself in a more rapid onset and greater intensity of symptoms. There were seldom more than one or 2 full 10 cc. samples after the intraventricular pilocarpine injections. These were usually laden with mucus and often bile-stained. This suggests that the pylorus under these conditions opens and permits the contracting stomach to eject its contents almost *en masse* into the duodenum.

Cardiospasm was an almost constant sequel of intraventricular pilocarpine injections as evinced by difficulty in passing the tube. The appearance of blood streaks in the subsequent sample was in many of these cases an indication of attendant trauma.

The other alimentary phenomena included retching and sometimes vomiting. Defecation was the rule, and the stools were often loose and copious indicating an increased motility of the whole gastrointestinal tract. Although mucus was frequently visible in the feces, gross blood was not apparent.

The general effects of pilocarpine injections, whether intraventricular or subcutaneous, include: (1) salivation, lacrimation, rhinorrhoea, and bronchorrhoea, with much mucus secretion; (2) sweating, especially of the palms, soles and scalp; (3) a warm flushed skin preceding the sweating, but thereafter a moist, cold, and clammy skin; (4) some pilo-erection; (5) a fall in rectal tem-

perature of 2°-3° C.; (6) a temporary reduction in pulse rate, perhaps to 70 or 80, as compared with the normal values which ran between 120 at rest and 180-200 (or more) during excitement and struggling. Sometimes the drop in pulse rate was not very marked or was very transient. In other cases it was striking and maintained. Usually the rate subsequently increased and often became irregular. Occasionally there were extrasystoles. The pulse, whether fast or slow was full and bounding; (7) a blood pressure (femoral) sometimes showing a definite fall and subsequently a slight rise coincident with the pulse variations; (8) markedly constricted pupils which failed to react; (9) considerable prostration, apathy, nausea, and anorexia.

After a moderate dose of pilocarpine the effects would begin to wear off in less than an hour. The animal became more active (and less tractable) and evinced thirst and later hunger, and was normal again in a few hours.

6471

Comparative Frequency of Peptic Ulcers After Deprivation of Bile and Pancreatic Juice.

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Berg and Jobling[1] reported the occurrence of peptic ulcers in dogs following the deprivation of bile. These findings were corroborated by Kim and Ivy[2] and Bollman and Mann.[3] The incidence of ulcers was approximately 60%.

During the past few years, in connection with other investigations, we have examined a number of animals deprived of their pancreatic secretions by means of fistulas, ligation of the pancreatic ducts, and pancreatectomy, and have been impressed by the infrequent occurrence of peptic ulcers in these animals compared to dogs in which bile was excluded. On the other hand, Elman and Hartmann[4] found ulcers in the duodenum in all of the animals of a series of 6 dogs

[1] Berg, B. N., and Jobling, J. W., *Arch. Surg.*, 1930, **20**, 997.
[2] Kim, M. S., and Ivy, A. C., *J. Am. Med. Assn.*, 1931, **97**, 1511.
[3] Bollman, I., and Mann, F. C., *Arch. Surg.*, 1932, **24**, 126.
[4] Elman, R., and Hartmann, A. F., *Arch. Surg.* 1931, **23**, 1030.

with pancreatic fistulas existing from 13 days to 18 days. They described the lesions as ulcers, although microscopic examination revealed only defects in the continuity of the mucosa, not involving the deeper muscular layers of the intestine. These investigators attributed the lesions to the loss of the neutralizing effect of pancreatic juice upon gastric acidity, and minimized the importance of bile as a factor in the development of peptic ulcers in dogs.

The following observations were made upon a series of 14 dogs deprived completely of pancreatic juice by means of fistulas made according to the technique of Rous and McMaster, as adapted by Elman and McCaughan.[5] Twelve of the animals received sodium chloride by mouth or intravenously; 2 received no special form of treatment and were killed after 25 days and 31 days respectively. Some of the animals were killed while they were still in good condition. Others developed symptoms typical of pancreatic insufficiency[6] and died spontaneously. The average daily output of pancreatic juice varied between 200 cc. and 700 cc. in different animals.

In 11 dogs, with drainage existing for 14, 18, 20, 20, 20, 20, 25, 25, 31, 37, and 40 days respectively, no changes in duodenum or stomach were present at autopsy. In 2 animals, multiple small superficial mucosal erosions were found after 16 days and 20 days respectively. In one dog 2 perforated duodenal ulcers were found after 25 days.

The fact that ulcers were encountered in only one of 14 dogs with fistulas is particularly significant since a larger number of animals was included in this series than in the experiments of Elman and Hartmann, and the periods of drainage were longer. We have found that dogs with pancreatic fistulas may be kept alive for long periods of time by providing for a suitable adjustment of the electrolyte equilibrium by means of sodium chloride.[7]

A great deal of confusion exists concerning the interpretation of the results of different investigators, because no distinction is made between simple erosions and real peptic ulcers. We do not include mucosal erosions within the category of ulcers, because similar lesions are encountered in dogs under a variety of circumstances, *viz.*, excessive vomiting, severe intoxications, infections, uremia, poisoning, bilateral adrenalectomy, and as a terminal phenomenon. Until more is known concerning the etiology and development of ulcers, we

[5] Elman, R., and McCaughan, J. M., *J. Exp. Med.*, 1927, **45**, 561.

[6] Berg, B. N., and Zucker, T. F., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 68.

[7] Zucker, T. F., Newburger, M. G., and Berg, B. N., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 666.

believe that it is better, especially in experimental studies, to restrict the term ulcer to defects which involve one or more muscular layers as well as the mucosa, and correspond to the acute or chronic penetrating or perforating lesions that are encountered in man.

The dog in which ulcers were found was one of 3 animals which developed jaundice, and showed marked degenerative changes in the liver at autopsy. This finding coincides with our earlier observations concerning the possible rôle played by biliary and hepatic factors in the genesis of peptic ulcers in dogs.[1] The occurrence of degenerative alterations in the liver after the prolonged deprivation of pancreatic juice has been described.[6]

In double pancreatic duct ligations, followed by atrophy of the pancreas (5 dogs), no changes in the duodenum or stomach were found, 23, 47, 53, 80, and 97 days respectively, after ligation. Ivy and Fauley[8] encountered ulcers in 6 out of 61 animals after ligation of the pancreatic ducts. We have not observed ulcers in dogs after total pancreatectomy, and others who have studied insulin treated depancreatized dogs (fed raw pancreas) over periods as long as 2½ years have not reported the occurrence of ulcers.[9]

Conclusion. The preponderance of evidence indicates that peptic ulcers develop in dogs more readily after the deprivation of bile than after the loss of pancreatic juice.

6472

Gastrointestinal pH in Rats as Determined by the Glass Electrode.*

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Investigators have reported many studies on gastrointestinal pH in animals, using various methods of determination—hydrogen electrodes, quinhydrone electrode, and colorimetry. The results have all been somewhat open to question because of the possible inaccuracy of these methods in a medium such as intestinal contents. For the

[8] Ivy, A. C., and Fauley, G. B., *Am. J. Surg.*, 1931, **11**, 531.

[9] Best, C. H., and Hershey, J. M., *J. Physiol.*, 1932, **75**, 49. Chaikoff, I. L., Macleod, J. J., Simpson, W. W., and Markowitz, J., *Am. J. Physiol.*, 1926, **76**, 210.

* This work was aided by a research grant from The Chemical Foundation to this department.

range of pH encountered in such work, the glass electrode seems to be the method of choice. We have used glass electrodes prepared by the methods of MacInnes and Dole[1] or Robertson.[2] The electrical apparatus developed for this work was that described by Rosebury.[3]

Four hundred and fifty-five albino rats were used. Groups were placed on various diets: (I) a "normal" diet which included corn, wheat, barley, oats, soy bean, milk, meat scrap, alfalfa, NaCl, and $CaCO_3$; (II) diet I + 10% lard; (III) diet I + 30% lard; (IV) bread; (V) potato; (VI) suet; (VII) lean beef; (VIII) McCollum's[4] rachitic diet 3143; (IX) diet VIII + 2% cod liver oil; (X) Steenbock's[5] diet 2965; (XI) diet X + 2% cod liver oil; (XII) Zucker's[6] diet 401; (XIII) diet XII + 2% cod liver oil; (XIV) diet XII + 15% cod liver oil. The rats on the last 3 and the suet diets showed consistent malnutrition.

The rats were killed by a blow on the head or by chloroform, and the abdomen opened immediately. The stomach, the small intestine in 3 approximately equal lengths, the cecum, and the colon

TABLE I.

Diet	No. Rats	Age	Stomach		Sm.Int. Upper Third		Sm.Int. Middle Third		Sm.Int. Lower Third		Cecum		Colon	
			av.	σ	av.	σ	av.	σ	av.	σ	av.	σ	av.	σ
Normal	64	1-1½ yr.	3.6	0.6	5.9	0.5	6.3	0.2	6.8	0.3	6.2	0.3	6.6	0.3
" + 10% lard	50	"	3.4	0.6	6.2	0.2	6.4	0.2	6.8	0.2	6.3	0.3	6.5	0.2
" + 30% lard	8	"	3.4	0.7	5.4	0.6	5.8	0.4	6.4	0.4	5.8	0.4	6.1	0.4
Bread	50	"	3.3	0.7	6.3	0.5	6.7	0.2	7.0	0.2	6.4	0.3	6.1	0.3
Potato	11	"	3.6	0.7	6.0	0.6	6.8	0.2	7.0	0.2	6.5	0.3	6.6	0.3
Suet	9	"	3.7	1.0	6.0	0.9	6.5	0.3	7.2	0.3	7.0	0.2	6.8	0.3
Meat	7	"	3.7	1.0	5.9	0.3	6.4	0.2	6.8	0.3	6.2	0.3	6.4	0.3
McCollum 3163	50	"	3.9	0.7	6.5	0.2	6.8	0.2	7.3	0.2	6.9	0.3	7.1	0.2
" + 2% C.L.O.	50	"	3.9	0.7	6.5	0.2	6.7	0.2	7.2	0.2	6.7	0.2	6.9	0.2
Normal	25	3 wk.	3.9	0.7	6.4	0.3	6.7	0.3	7.1	0.3	7.0	0.3	7.0	0.4
McCollum 3143	35	"	4.8	1.0	6.5	0.3	7.0	0.3	7.5	0.5	7.3	0.3	7.4	0.3
" + 2% C.L.O	23	"	4.6	0.7	6.3	0.4	6.8	0.3	7.4	0.4	7.1	0.2	7.1	0.2
Steenbock 2965	23	"	3.9	1.0	6.4	0.5	6.6	0.5	7.0	0.3	7.0	0.3	7.1	0.3
" + 2% C.L.O.	13	"	4.3	0.4	6.6	0.2	6.5	0.3	6.9	0.2	6.9	0.2	7.0	0.1
Zucker 401	17	"	4.6	0.6	6.6	0.2	6.7	0.1	7.0	0.2	7.0	0.2	7.2	0.2
" + 2% C.L.O.	13	"	4.5	0.5	6.5	0.1	6.7	0.1	6.9	0.1	6.9	0.1	7.0	0.1
" + 15% C.L.O.	7	"	4.9	0.5	7.1	0.2	7.1	0.2	7.2	0.3	7.1	0.3	7.1	0.2
Total, adult	299	1-1½ yr.	3.6	0.7	6.1	0.5	6.6	0.3	7.0	0.3	6.4	0.4	6.6	0.5
" young	156	3 wk.	4.3	0.9	6.5	0.4	6.8	0.3	7.2	0.4	7.1	0.3	7.1	0.3
" all rats	455		3.8	1.1	6.2	0.5	6.7	0.3	7.1	0.3	6.7	0.5	6.8	0.5

[1] MacInnes, D. A., and Dole, M., *J. Am. Chem. Soc.*, 1930, **52**, 29.

[2] Robertson, G. R., *J. Ind. Eng. Chem., An. Ed.*, 1931, **3**, 5.

[3] Rosebury, F., *J. Ind. Eng. Chem., An. Ed.*, 1932, **4**, 398.

[4] McCollum, E. V., *J. Biol. Chem.*, 1921, **47**, 50.

[5] Steenbock, H., *J. Biol. Chem.*, 1925, **64**, 263.

[6] Jephcott, H., and Bachrach, A. L., *Biochem. J.*, 1926, **20**, 1350.

were quickly tied off and removed and the contents gently expressed under oil. The contents were then diluted sufficiently with water and the pH determined.

Plotting histograms of the incidence of the data revealed that if calculated as pH the occurrences closely approximated the normal bell-shaped curve. We have, therefore, in using statistical formulae to describe the data, considered them in the pH form throughout rather than as C_H. The averages with their standard deviations are shown in the table.

For a given level of the tract, there is a very striking tendency to maintain a normal pH range in spite of wide variations in the nature of the diet. In general, the pH increases with descending levels to the ileocecal valve; the cecal contents are more acid than the ileum; and in the colon the pH again rises over that in the cecum. On the suet and the bread diets, the acidity of the colon was greater than that of the cecum.

The pH values for the young rats show a tendency to be slightly higher than for the adults. The individual series differences, while not surely significant statistically, nevertheless occur so consistently that the reality of a small age difference in pH becomes apparent.

The McCollum rachitogenic diet 3143 gave, in each section of the gut below the stomach, an increase of pH over the normal figures which was more than 2 times the probable error of the difference. When the consistency of the effect at all of the levels is considered, it appears that this is a real difference. The addition of cod liver oil to the diet caused a consistent though small return of the pH towards the normal.

The other diets, including the other rachitogenic diets, gave no divergences from the normal that can be interpreted as being surely significant statistically.

When the data for individual rats are studied, it appears that there is no great correlation between the recurrences of high or low pH values in consecutive levels of the tract. This implies, perhaps, that the local conditions in the gut (secretions and absorptions and bacterial flora) are more potent factors in determining the hydrogen ion concentration than is the pH of the food residues themselves as they pass down the lumen, although the question of the titratable acidity still remains to be investigated.

6473

Serum Proteins and Lipoids in Infantile Eczema.*

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A comparison was made of infants and children with eczema and an essentially normal group of similar age distribution without eczema or known allergic disturbance, in respect to the concentration in the serum of total protein, albumin, globulin, cholesterol and lipoid phosphorus. The protein determinations were made by the gravimetric method of Barnett, Jones and Cohn,[1] so modified as to permit the separate measurement of the albumin and globulin fractions, the latter by difference.† Cholesterol was determined by the method of Myers and Wardell.[2] The lipoid phosphorus was determined by a combination of the methods, slightly modified, of Bloor[3] (preliminary extraction), Benedict and Theis[4] (determination of phosphorus). The protein osmotic pressure was calculated from the figures for albumin and globulin.

There were 31 children in the eczema group and 25 in the control group. The results—excluding lipoid phosphorus in which no differences of interest were found in the two groups—can be summarized by giving the mean, the 10 percentile and 90 percentile values, in order.

1. Total protein. Eczema: 7.13; 6.13; 7.95 (gm. %). Controls: 6.82; 6.01; 7.73.

2. Albumin. Eczema: 5.29; 4.62; 5.93. Controls: 4.72; 4.05; 5.37.

3. Globulin. Eczema: 1.84; 1.26; 2.66. Controls: 2.10; 1.25; 3.17.

4. Albumin: globulin ratio. Eczema: 3.19; 1.96; 4.14. Controls. 2.52; 1.45; 3.77.

* This work was made possible by a grant from the Rockefeller Fluid Research Fund.

[1] Barnett, C. W., Jones, R. B., and Cohn, R. B., *J. Exp. Med.*, 1932, **55**, 683.

† The globulin was also determined directly, and fair agreement obtained with the figures by difference. The absolute amounts of globulin in the small samples of blood available were low and we believe that the estimation by difference is somewhat more accurate.

[2] Myers, V. C., and Wardell, E. L., *J. Biol. Chem.*, 1918, **36**, 147.

[3] Bloor, W. R., *J. Biol. Chem.*, 1918, **36**, 33.

[4] Benedict, S. R., and Theis, R. C., *J. Biol. Chem.*, 1924, **61**, 63.

5. Protein osmotic pressure. Eczema: 32.2; 28.0; 35.9 (mm. Hg.). Controls: 29.0; 25.3; 32.8.

6. Cholesterol. Eczema: 195.7; 187; 139 (mgm. %). Controls: 173.1; 132; 194.

The more striking differences observed were: (1) Marked elevation of the serum albumin, albumin-globulin ratio, and protein osmotic (oncotic) pressure in the serum of the eczema group; (2) Marked elevation of the serum cholesterol in the eczema group, 40% of which showed figures in excess of 200 mg. %; (3) Slight, possibly not significant, lowering of the serum globulin in the eczema group; (4) Moderate elevation of the total protein in the eczema group, due mainly to the high albumin fraction; (5) No difference between the 2 groups in respect to lipoid phosphorus.

The exact significance of the differences noted is not clear. All the children were receiving milk in considerable quantities. In a few instances, precipitin tests for bovine lactalbumin were made on the sera and positive results obtained but these occurred in the controls as well as in the patients with eczema. Even assuming that the increase in serum albumin was wholly due to absorption of undigested lactalbumin—an assumption that is not supported by convincing proof—this might be the result either of increased absorption or from failure to dispose of a normal amount of absorbed protein at a normal rate.

In any case, it may be pointed out that a definite increase, such as has been observed here, in the albumin fraction of human serum is an unusual phenomenon.

6474

Experimental Granulopenia.

E. WESTERVELT DENNIS. (Introduced by John F. Kessel.)

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The etiology of so-called "agranulocytosis"[1] has not been explained. Kracke[2] has recently produced the disease in rabbits by repeated small injections of benzene, but there is little evidence that such a substance is responsible for the syndrome in man. However,

[1] Schultz, *Deutsche med. Eschnschr.*, 1922, **48**, 1495.

[2] Kracke, *Am. J. Clin. Path.*, 1932, **2**, 11.

certain of the pyogenic bacteria are capable of producing *leucocidin* which is specific and highly lethal for granulocytes. One or more of these organisms is almost constantly associated with focal infections, which in turn are commonly accompanied by leucopenia.[3] A history of focal infection can be elicited from most of the reported cases of agranulocytosis, and their possibility cannot be excluded from the remainder.

Virulent cultures of *Staphylococcus aureus, Streptococcus hemolyticus, Streptococcus viridans,* and a species *Proteus,* the latter being isolated from the blood and organs of a fatal case of granulopenia, were each sealed into parchment capsules of 5 cc. capacity,[4] and the capsules were placed aseptically in the abdominal cavity of rabbits. Total and differential leucocyte counts were made twice daily both before and after operation. The experiment was controlled by counts on the blood of rabbits which received capsules of sterile broth. The behaviour of the neutrophils is shown by morning

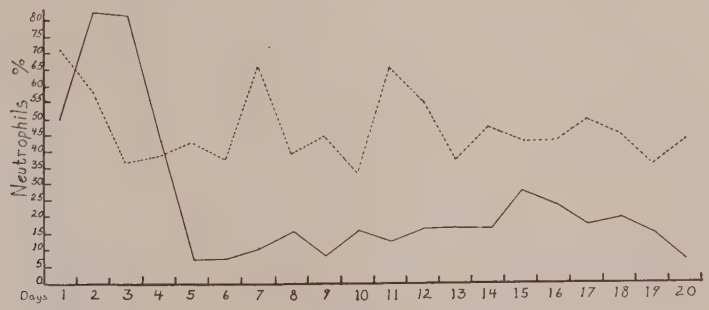

FIG. 1.
Neutropenia produced by *Staphylococcus aureus.*

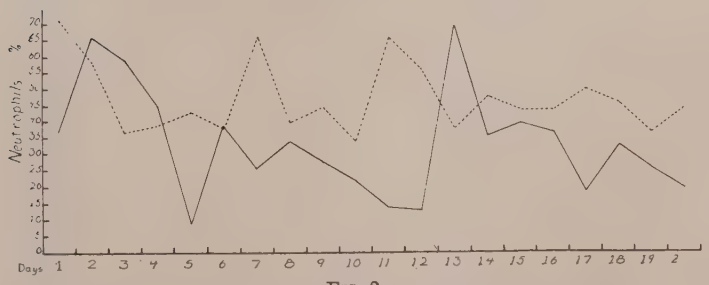

FIG. 2.
The effect of *Streptococcus hemolyticus* on the neutrophils.

[3] Appleton, Bacterial Infection (Lea and Febiger, Philadelphia), 1925, 113.
[4] Dennis, *Science,* in press.

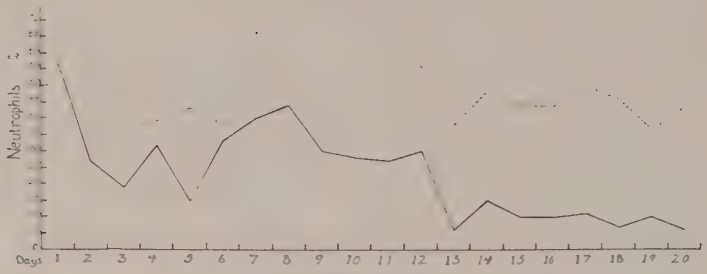

FIG. 3.
Neutropenia produced by *Proteus syriensis* isolated from a case of agranulocytosis.

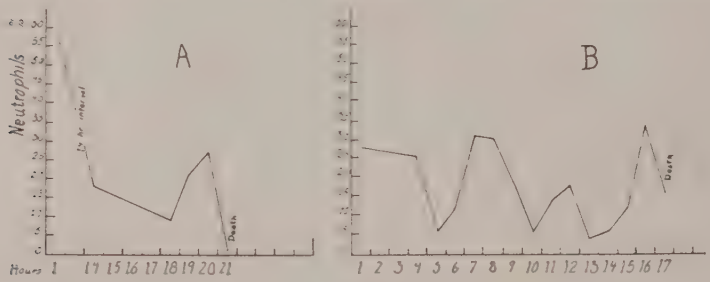

FIG. 4.
Acute leucopenia produced in two rabbits (A and B) by means of *Streptococcus viridans*.

blood counts represented in the accompanying charts, the dotted lines representing the control animals and the solid lines the experimental animals. It is of particular interest that the *S. viridans* was essentially avirulent when injected directly into an animal, yet the results were startling when the above method was used. Both rabbits receiving *S. viridans* had a terminal pneumococcic septicemia.

Conclusions. Under conditions simulating a chronic focal infection, *S. aureus*, *S. hemolyticus*, and *Proteus sp.* are capable of inducing a marked neutropenia which has been sustained for 5 months in animals implanted with Staphylococcus and Proteus. By this method *S. viridans* exhibits a virulence that is not manifested by direct inoculation. It shows a greater toxicity for the bone marrow and the entire leucopoietic system, and the picture produced in rabbits is the same as that of acute "agranulocytosis" in man. The pathogenic factor is a diffusible toxic substance, the specificity of which indicates that it is leucocidin.

6475

Impulses in Cardiac Sympathetic Nerves.*

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A better understanding of the mechanism of the reflex regulation of heart rate and blood pressure is to be gained from a study of the afferent nerve impulses from the circulatory system and of the efferent impulses to the heart and blood vessels. Previous communications have described the nature of the nervous discharge from the arch of the aorta and from the carotid sinus,[1, 2] and its relation to blood pressure. On the efferent side, sympathetic impulses concerned in maintaining the tone of the blood vessels have been recorded.[3] The present report is concerned with the activity of the sympathetic fibers to the heart.

One of the small nerve twigs running to the heart from the stellate or inferior cervical ganglion in a cat under urethane anesthesia was freed from the surrounding tissue and cut close to the heart, all other cardiac sympathetic fibers remaining intact. The nerve was then slung onto electrodes and the action potentials, after amplification, were recorded by means of an oscillograph. Figure 1A shows a typical discharge. It will be observed that the impulses tend to come in bursts but in general we have not been able to identify the frequency of these volleys with the heart rate or respiratory rhythm, although it has been shown[3] that in the case of sympathetic nerves carrying fibers to the blood vessels, there is usually a grouping that is synchronous with either the respiratory or cardiac rhythm. The records further show that under the conditions of these experiments there is normally a "tonic" sympathetic discharge to the heart.

The relation of the discharge in the cardiac sympathetic fibers to the heart rate is shown by a comparison of Figures 1A and B. In A, the frequency of the heart beat was 132 per min. Adrenalin was then injected intravenously, resulting in a complete cessation

* The expenses of this research have been in part defrayed by a grant from the Committee on Scientific Research of the American Medical Association.

† Associate in surgery.

1 Bronk, D. W., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 1014.

2 Bronk, D. W., and Stella, G., *J. Cell and Comp. Physiol.*, 1932, **1**, 113.

3 Adrian, E. D., Bronk, D. W., and Phillips, G., *J. Physiol.*, 1932, **74**, 115.

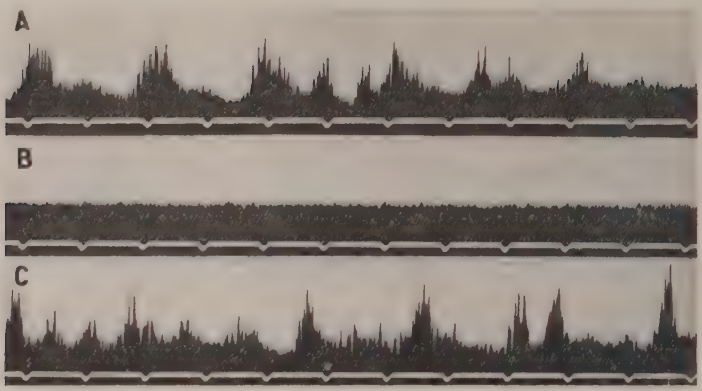

FIG. 1.

Action potentials in a cardiac branch of the sympathetic. A. Heart rate 132 per min. B. Following intravenous injection of adrenalin. Impulse discharge abolished. Heart rate 80 per min. C. Later. Blood pressure down to normal. Heart rate 130 per min. White marker gives time in 1/5 sec.

of the sympathetic impulses as shown in B. Associated with this abolition of impulses there was a decrease in the heart rate to 80 per min. Some minutes later, the sympathetic discharge had returned as in C and the frequency of the heart beat was again about 130 per min. Inhalation of amyl nitrite on the other hand increased the activity of these sympathetic fibers and was associated with cardiac acceleration. There is then a definite relation between the number of sympathetic impulses in the cardiac fibers and the heart rate. In general, any agent producing an increase in blood pressure causes a decrease in the discharge in cardiac sympathetic fibers, whereas a decrease in blood pressure is followed by an increase in the discharge provided the afferent nerves from the carotid sinus and aorta are still functional. These results are analogous to those obtained by Adrian, Bronk and Phillips[3] on sympathetic fibers to the blood vessels. The direct action of various chemical agents on the centers is being investigated.

Records of action potentials in practically any of the cardiac branches of the vagus have shown a similar type of nervous discharge and a similar relation to heart rate. Subsequent section of the ascending branches of the stellate ganglion have, however, abolished the impulses. It may be concluded, therefore, that vagal branches going to the heart carry a considerable number of sympathetic fibers which conduct impulses that are concerned with cardiac acceleration. The nature of the discharge in vagal fibers associated with cardiac inhibition will be discussed in a subsequent paper.

6476

Cornification and Molting in Triturus.

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Marked variations in intervals between molts and in duration of single molts were observed in 33 normal adult *Triturus viridescens* kept for 16 weeks in individual finger bowls in the laboratory after a previous laboratory residence of 3½ months. One animal did not molt until the eleventh week of observation; another shed 3 times with an interval of 8 weeks between the first and second molts and 5 weeks between the second and third; another molted 5 times in 6 weeks and then not again for 4 weeks. The average number of molts for the 33 animals was 4.97 in the 16 weeks, ranging from one to 9. The average interval between successive molts (end of one molt to beginning of next) was 13.19 days, with a range of 0 to 59 days. Single molts lasted from one day (1/5 of all recorded molts) to 28 days, and averaged 7.8 days. During the 16 weeks, the group was molting about 34.8% of the time, a higher frequency than that recorded by Adolph and Collins,[1] whose animals molted 9% of the time after being in captivity for 3 months and 22% of the time after 5 months, but consistent with a longer laboratory confinement (7½ months).

These data seem to throw light on the variability in the degree of blackening of thyroidectomized animals which results from the continued cornification of epidermis in such animals without its being shed. Of a group of 362 thyroidectomized animals, 243 became very black and at autopsy all but 33 lacked thyroid tissue. These possessed a few follicles usually located in the median line. Among the 119 that did not blacken after thyroid removal, no thyroid tissue was demonstrable by dissection in 9, but the rest had from one to 10 follicles. Occasional animals that blackened and then molted some time after the operation invariably had thyroid tissue. These variations, in which some animals with small amounts of thyroid gland blackened and others with apparently none or very little blackened faintly or not at all, seem correlated with the above described differences in the molting records of normals. Both sets of data indicate that the rate of cornification is an individual matter. The data on the thyroidectomized group further indicate that cornifica-

[1] Adolph, E. F., and Collins, H. H., *J. Morph.*, 1925, **40**, 575.

tion proceeds independently of the amount of thyroid secretion. However, it appears that the thyroid hormone controls the molting mechanism since molting of thyroidectomized or hypophysectomized animals or ones lacking both glands can only be brought about by administering (or releasing in the case of hypophysectomized individuals) thyroid hormone or iodine.[2]

Possible factors in the molting mechanism were tested by using both normal and thyroidectomized animals. Thyroxin was used to induce the molt in thyroidectomized animals; pilocarpine and atropine to release and prevent the release, respectively, of cutaneous gland secretion; sodium nitrite and ephedrine or epinephrine to increase or decrease, respectively, the lymph supply to the skin.[3] Salt solution was injected into control animals or they were untreated.

1. *Histological study of skin.* No striking changes were observed in the skin of thyroidectomized animals killed during the latent period before the beginning of a molt induced by an injection of thyroxin (0.1 cc. of a 0.01% solution, Squibb). When the molting reaction is becoming evident (72 to 84 hours after the injection), there are a few more mitotic figures in the stratum germinativum, but the proliferation is so slight that it does not seem likely to play a significant rôle in shoving the cornified layers off or in severing them from the uncornified layers beneath. Further, the tardiness in the appearance of the mitoses suggests that the thyroid hormone does not stimulate the skin directly, but initiates a chain of reactions in the body that lead to molting (Speidel).[4]

2. *Animals in moist chambers.* Seven of 12 normal animals placed in moist chambers molted in 28 to 96 hours after confinement without any type of injection. Exposure to moist air was also followed by shedding in ½ to ⅔ of the normal animals injected with each solution. However, since uninjected ones molted with equal frequency, it is concluded that the shift from water to moist air rather than the type of injection was the chief factor in the molting of the injected animals. Further, since uninjected and injected thyroidless newts, *with the exception of thyroxin-injected ones,* failed to molt whether in water or moist air, it seems plausible that the thy-

[2] Adams, A. E., and Richards, L., *Anat. Rec.*, 1929, **44**, 222; Adams, A. E., Richards, L., and Kuder, A., *Science*, 1930, **72**, 323; Adams, A. E., *Anat. Rec.*, 1931, **51**, Suppl. 49; Adams, A. E., Kuder, A., and Richards, L., *J. Exp. Zool.*, 1932, **63**, 1.

[3] Hatcher, R. A., and Eggleston, C., Useful Drugs, Eighth Edit., 1930, Am. Med. Assn., Chicago.

[4] Speidel, C. C., *J. Morph.*, 1926, **43**, 57.

roid gland is involved in the normal molting mechanism. Confinement of thyroxin-injected thyroidectomized individuals in moist chambers lengthened the latent period before the induced molt became evident (compare an average latent period of 72.5 hours for 7 such animals in water with one of 86.5 hours for 14 in moist chambers). These results show that a water environment is not necessary for molting, but probably facilitates the loosening of the cornified cells.

3. *Stimulating or depressing the cutaneous gland action.* It seems unlikely that the cutaneous glands have a rôle in molting because their ducts open to the surface of the epidermis, even in thyroidectomized animals with 4 or more cornified layers. Moreover, normal or thyroidless animals in moist chambers often show collapsed skin glands. Stimulation of the glands of 23 thyroidectomized newts by single injections (0.1 cc.) of a 1% pilocarpine hydrochloride solution produced no molting although within an hour the secretion was abundant on the surface of the skin. Ten of the animals were observed for 76 hours, 13 for 14 to 21 days. Pilocarpine also failed to speed up molting when it was given with thyroxin (0.01% solution, Squibb) to 5 athyroid newts. Furthermore, a 0.1% solution of atropine sulphate when administered with thyroxin did not delay the molt in 10 thyroidectomized animals, when compared with that occurring in animals injected with thyroxin only. This evidence, therefore, rules out the discharge of cutaneous gland secretion as involved in the chain of reactions causing molting.

4. *Varying the cutaneous lymph supply.* Since "red phase" (terrestrial) and "green phase" (aquatic) *Triturus* that molted in moist chambers had dry skin surfaces, but had fluid under the cornified layers, it seemed likely that the cutaneous lymph supply might be linked with the molting phenomena. Further, intercellular spaces in the lower epidermal layers are prominent and there is a marked capillary bed at the dermal-epidermal border. Therefore, sodium nitrite injections (a single injection, 0.1 cc., of a 1% or two injections of a 0.01% solution daily for 14 days) were used to relax the blood vessels and to allow more lymph to flow out into the skin.[3] However, this treatment failed to induce a molt in 25 thyroidectomized animals observed from 3 to 21 days. Although 10 of these were observed only 76 hours, the remaining 15 died or were killed from 5 to 21 days after treatment had begun, that is, well beyond the usual latent period for induced molts. Neither did sodium nitrite when given with thyroxin hasten the appearance of the induced molt in 5 thyroidless animals. Injections of ephedrine hydrochloride (a single injection, 0.1 cc., of a 1% solution or 2 injec-

tions of a 0.01% solution daily for 14 days) or of epinephrine (a single injection, 0.1 cc., of a 0.1% solution or 2 injections of a 0.1% solution daily for 8 days) were given to decrease the lymph supply to the skin. As was to be expected, no molts were induced in 10 thyroidectomized animals given the ephedrine or in 15 given epinephrine. Of 17 normals injected with these drugs, all but 4 individuals molted without any noticeable delay, but considering the variation of normal individuals in water and in moist chambers, these data do not give conclusive evidence that decreasing the lymph supply in normals retards their molting. On the other hand, when injections of ephedrine or epinephrine were given at the same time as thyroxin to thyroidless animals, the molt was retarded when compared with that of thyroidectomized animals receiving thyroxin only. For example, in one experiment, the average latent period for 4 thyroxin-injected animals was 115 hours, while for 9 of 15 animals receiving ephedrine or epinephrine with the thyroxin, it was 118 hours. Five of the remaining 6 molted much later (6, 7, 12, 14, 14 days after treatment had begun) and one did not molt although observed for 33 days. This failure to react after thyroxin is unique among large numbers of such tests carried out in this laboratory. In another experiment, the period before molting was observed in 10 animals receiving ephedrine with thyroxin was 14.5 days as compared with 78.8 hours for 5 animals receiving thyroxin only. These experiments seem to implicate the circulatory system in the chain of reactions leading to the actual molt (Speidel[4]).

Conclusion. The rate of cornification of the superficial epidermis of *Triturus* is markedly variable and probably independent of thyroid control, but the molting of the cornified layers is dependent on the thyroid. Experiments indicate that a change in cutaneous circulatory conditions is probably an essential factor in the molting mechanism.

6477

Apparent Increased Resistance of Vitamin B-Deficient Rats to an Acute Infection.

L. REINER AND J. B. PATON. (Introduced by C. S. Leonard.)

From the Burroughs Wellcome and Co. Experimental Research Laboratories, Tuckahoe, N. Y.

Most recent investigations emphasize the fact that avitaminosis tends to decrease the resistance to infection. However, in a long series of experiments on trypanosome infection of rats (*Trypanosoma equiperdum*) just the opposite effect was produced. The lack of vitamin B complex appeared to increase the resistance. This unexpected phenomenon seems to be worthy of note although a satisfactory explanation has not yet been found.*

The effect was first noticed when one group of infected experimental animals was accidentally kept on a diet low in vitamin B complex and died irregularly and somewhat later than the controls. In further experiments rats were kept on a diet deficient in B complex for as long a period of time as possible. They were then infected with trypanosomes and control rats, receiving the same diet made complete by addition of the vitamin B complex, were infected at the same time with the same emulsion of trypanosomes. The controls survived the infection with a few hundred thousand trypanosomes approximately 4 days and died all within a half day interval. The infected vitamin-deficient rats lived one, 2, or even 6 days longer. No difference was found in the development of the disease since the trypanosomes appeared at the same time and multiplied apparently at the same rate in the controls and in the vitamin B complex deficient rats, but the latter lived for a comparatively long time with a great number of trypanosomes in the blood stream; this number, however, did not surpass that found in the blood of controls at the time of their death.

Rats kept on a vitamin B_1 free diet (recommended by the U.S.P. Vitamin Conference, January, 1932) but receiving vitamin G (B_2) in the form of autoclaved yeast, showed the increased survival almost to the same extent as those which received neither vitamin B_1 or G. Those which received rice polishings extract (B_1) but no autoclaved yeast (G) occasionally lived longer than the controls. If

* Solazzo (*Z. f. Immunitäts.*, 1929, **60**, 239) claims to have lowered the resistance of pigeons against *Trypanosoma brucei* by keeping them on a diet deficient in vitamin B and in an environment of low temperature.

the latter effect was not accidental, then vitamins B_1 and G may be considered, at least from the point of view of the phenomenon discussed in this paper, as "related" vitamins.

It is obvious that in a large group of rats kept on vitamin B deficient diet all will not have reached the same degree of depletion at a definite time (the time of inoculation†) and hence it was not to be expected that all would show uniform effects of avitaminosis on trypanosome infection. Therefore, the statistical average of the survival of the vitamin-deficient animals subtracted from that of the controls was taken as a measure of the effect observed. This difference was 0.99 days with a probable error of ± 0.07 days,‡ an average value which was obtained from the results of 11 independent experiments comprising 165 rats, 108 of which were deficient in vitamin and 57 were controls. The number of trypanosomes injected constituted the only difference between the various experiments. Other experiments with over a hundred rats gave essentially identical results but these were not used in calculating the above averages because the experimental conditions were varied. The data from a typical experiment are given in the table.

A few experiments have been made attempting to explain the effects described. Although they did not solve the problem, they do exclude some explanations which one would have supposed were the most likely. We first thought that the protozoa depend upon the vitamin content of the rat organism, just as much as do the cells of the host itself. However, feeding of the vitamin from the time of inoculation until death did not noticeably change the course of the infection, although the symptoms of the deficiency (polyneuritis) improved even during the first day.

In one experiment a group of control rats was kept starving. The trypanosomes appeared in the blood stream somewhat later than in the animals kept either on deficient or complete diets. These rats died of starvation before the infection was fully developed. This may indicate that starvation also affects the course of the infection but in a different way from the lack of vitamin B complex; more data are required to settle this point definitely.

Moreover, animals kept on a diet deficient in vitamin A may be

† The rats were inoculated when it was believed from previous experience that the most depleted rats could not survive longer than the expected course of the infection.

‡ Thus the observed effect is 14.1 times greater than its probable error and by Gauss' Law of Error the probability that the effect is real and not due to error is greater than 10^{10} to 1. Rietz and Mitchell, *J. Biol. Chem.*, 1910, **8**, 297.

TABLE I.

Effect on Trypanosomiasis of Diet Free from Vitamin B Complex or Components.

No.	Av. Wt. in gm. Initial	Final	% Gain or Loss	Depletion Period Days	Days After Inoculation, No. Rats Which Died 4	4½	5	6	6½	7	7½	8½	10½	Av. Time Survival Days	Stand. Devia.	Av. Time of Surviving Controls	% Rats Surviving Controls
840 845 3 ♂, 3 ♀ B_1 (no G)	36.8	81	+120	42	3				1		1	1		6.25	1.91	1.75	50
852 857 3 ♂, 3 ♀ G (no B_1)	38.5	96.5	+150	42		3	1	1	1					5.1	0.80	0.6	50
861-866 3 ♂, 3 ♀ No B_1 or G	59.3	51.3	— 13.4	20*		4	1				1			5.08	1.10	0.58	33
891-896 3 ♂,† 3 ♀ No B_1 or G	46	43.8	— 5	20	1		1	1		1			1	6.6	2.27	2.1	80
834 839 3 ♂, 3 ♀ Controls	34.3	131.3	+282			6								4.5	0.25		

* Started later because deprived of both B and G.

† One died before inoculation.

considered just as much in a state of starvation as those deprived of vitamin B, yet rats lacking vitamin A were found to die even earlier in trypanosomiasis than those on a complete diet as far as can be judged by experiments on a small group of animals (7).

The methylene blue reduction rate of trypanosomes suspended in the blood serum of B deficient animals was the same as the rate of those suspended in normal rat serum.

Finally, mechanical factors were considered. The viscosity of the blood of B deficient rats is increased and the entire organism is dehydrated. It is conceivable that this damages the vitality of the trypanosomes. However, the medium in which the trypanosomes move is really the plasma and therefore the viscosity of the plasma rather than that of the entire blood is the factor which may influence their motility. The citrate plasmas of the 2 B deficient animals tested did not have a higher viscosity than that of the controls.

These experiments suggest that the delayed death of vitamin B deficient rats infected with trypanosomes may be due to some secondary change in the intermediary metabolism of these deficient animals.

Summary. 1. Albino rats kept on vitamin B complex-free diet survived on an average longer after infection with *Trypanosoma equiperdum* than controls kept on a complete diet. 2. Lack of vitamin B_1 seems to be the important factor although a slight effect was also observed with animals receiving B_1 but no G. 3. No change was found in the plasma of deficient animals which could account for the phenomenon observed. 4. Although feeding of vitamin B, after the injection of trypanosomes cured polyneuritis it did not restore the normal reactivity towards the infection before death. 5. Rats kept on a vitamin A free diet and infected with *Trypanosoma equiperdum* apparently died somewhat earlier than controls kept on a complete diet.

6478

Precocious Development of Sexual Characters in the Fowl by Daily Injections of Hebin. I. The Male.*

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In a previous report the precocious development of sexual characters following homeoplastic hypophyseal implants in cockerels was discussed.[1] The present report concerns the effects of daily administrations of hebin, a purified gonad-stimulating hormone prepared from sheep pituitary glands, on the juvenile sex characters of cockerels.[2]

Light brown Leghorn cockerels ranging from 21 to 47 days at the beginning of the experiment received daily injections of hebin over a period ranging from 14 to 36 days. The daily dosages administered per bird varied from 4 to 32 rat units. Treated and control birds were weighed and head furnishings measured at regular intervals. Treated individuals, with but one exception, remained active and in good condition throughout the duration of the experiments.

The first effect to be noticed was a pronounced stimulation of head furnishings, in some instances, as early as 48 hours after injections began. These became turgid and reddish in color and revealed steady growth throughout so that at the conclusion of experiments they were usually perceptibly larger than controls—in some instances very considerably so. Bird No. 250, 28 days old when the experiment began, received 20 rat units daily for 21 days. The day the experiment began its comb measured 2.0^{-} cm. in length and 0.8^{+} cm. in height, while 21 days later its comb measured 7.3 cm. in length and 3.7 cm. in height. The largest control comb measured but 4.3 cm. in length and 1.8 cm. in height at the conclusion of the experiment, though its size at the beginning was similar to that of the treated individual. Some of the older individuals were known to crow and attempt treading, a reaction not

* This investigation was supported in part by a grant from the Committee for Research in Problems of Sex of the National Research Council; grant administered by Prof. Frank R. Lillie.

1 Domm, L. V., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 308.

2 For similar experiments with females see Domm, PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 351.

exhibited by normals until considerably older.† Plumage and spurs revealed no noticeable modifications.

Necropsy revealed hypertrophy of testes which were larger and heavier in treated individuals. The testes of bird No. 250 (See above) weighed 0.669 gm. whereas those of 2 controls weighed 0.176 and 0.116 gm. respectively. The ductus deferens revealed hypertrophy following administration of higher concentrations. Thyroids were likewise larger and heavier in treated individuals. These in bird No. 250 weighed 0.123 gm. whereas those of 2 controls weighed but 0.031 and 0.016 gm. respectively. Such organs as the spleen, liver, and heart did not seem to show any significant changes in weight, in fact, experimental spleens and livers were quite frequently somewhat lighter than respective controls.

Histologically testes from treated individuals revealed significant modifications. The tubules of control testes were in all instances distinctly juvenile, many still showing primordial germ cells, whereas those of treated testes were greatly advanced. In all of the latter the tubules were distinctly larger and revealed spermatogenesis.

The results in general confirm the earlier observations of Domm[3] on the precocious development of sexual characters in Leghorn cockerels following daily subcutaneous homeoplastic hypophyseal implants. However, head furnishings revealed a greater response and thyroids hypertrophied only following injections, probably a quantitative response attributable to a larger amount of hypophyseal hormone having been supplied by the injections. Preliminary experiments on capons revealed no response in head furnishings or behavior though they did reveal hypertrophy of thyroids.

It is assumed that the injected gonad-stimulating hormone acted directly on the thyroids and gonads and that the precocious development of the latter is responsible for the development of other sexual characters.

† Young males have recently been found to crow at 9 days of age following 6 daily injections, while the initial treading reactions were first noticed at 13 days following 10 daily injections. Treatments began when 3 days old.

6479

Precocious Development of Sexual Characters in the Fowl by Daily Injections of Hebin. II. The Female.*

L. V. DOMM AND H. B. VAN DYKE.

From the Whitman Laboratory of Experimental Zoology, and the Department of Pharmacology, University of Chicago.

The precocious development of sexual characters following homeoplastic hypophyseal implants in juvenile Leghorn females was previously discussed by Domm.[1] In a second series the effects of daily subcutaneous injections of hebin on these characters were studied.[2]

Light brown Leghorn females ranging from 21 to 47 days at the beginning of the experiment received daily injections of hebin over a period ranging from 14 to 36 days. The daily dosages administered per bird varied from 4 to 32 rat units. Head furnishings were measured and birds weighed at regular intervals as in experiments on cockerels. All treated females remained active and in good condition. In none of the experiments did there seem to be a significant difference in weight between treated and control, both groups showing consistent gains throughout.

Here, as in the male, the first effect to be noticed was a phenomenal growth of head furnishings. This was definitely noticeable within 48 hours in individuals receiving higher concentrations. In such experiments the head furnishings revealed a continuous high rate of growth throughout, becoming large and masculine in character. Bird No. 249, 28 days old when the experiment began, received 20 rat units daily for 21 days and was killed on the day following the last injection. Its comb measured 1.7 cm. in length and 0.5 cm. in height when experiment began and 4.5 cm. by 2.3 cm. on the day the bird was killed. The best control increased from 1.3 cm. length and 0.4 cm. height to 2.1 cm. by 0.7 cm. during this period. The growth of head furnishings in treated females frequently approximates that of similarly treated males. The comb in such individuals is stout of blade and erect whereas that of similar size in

* This investigation was supported in part by a grant from the Committee for Research in Problems of Sex of the National Research Council; grant administered by Prof. Frank R. Lillie.

[1] Domm, L. V., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 310.

[2] For similar experiments with cockerels see Domm, PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 349.

normals, only found in much older individuals, shows a thin lobby blade. Plumage, spurs, and behavior were apparently unaffected.

Postmortem revealed considerable hypertrophy of ovaries. These were larger and heavier than controls but showed no indications of ovulation. The ovary of bird No. 249 (See above) weighed 0.466 gm. while those of 2 controls weighed 0.089 and 0.090 gm. respectively. The oviducts likewise showed an astonishing hypertrophy comparable to that normally preceding ovulation. That of bird No. 249 weighed 0.696 gm. whereas those of 2 controls weighed but 0.042 and 0.041 gm. respectively. Thyroid weights showed similar differences in favor of treated birds. These in No. 249 weighed 0.122 gm. while those of 2 controls weighed but 0.011 and 0.019 gm. respectively. Differences in weights of liver, spleen, and heart were probably not significant, though curiously experimental livers and spleens were usually heavier, whereas the converse was generally found in the male. Rudimentary right gonads and Wolffian ducts hypertrophied.

Preliminary histological studies seem to show a greater abundance of interfollicular tissue and somewhat larger follicles in experimental ovaries. Sections of treated and control oviducts, however, revealed striking differences. Normals showed low mucous folds devoid of tubular glands and conspicuous muscle layer whereas experimentals showed high mucous folds, well developed tubular glands and conspicuous peripheral muscle layer.

The results confirm the earlier observations of Domm[1] following daily hypophyseal implants on juvenile Leghorn females. However, as in the male, certain characters showed a greater response following injections. This is particularly true of head furnishings though it probably applies to other characters as well. Here also, thyroids showed perceptible hypertrophy only following injections, which probably signifies that the injections have supplied a greater quantity of hypophyseal hormone. Preliminary tests on young sinistrally ovariotomized Leghorns revealed hypertrophy of thyroids, rudimentary right gonad and Wolffian ducts, and some growth of head furnishings.

It is assumed that the injected gonad-stimulating hormone acted directly on thyroids and gonads, that its stimulation of the latter caused a precocious endocrine, rather than gametogenetic, functioning, which in turn is responsible for the development of other sexual characters. It is further assumed that the male hormone liberated by the stimulated medullary tissue is responsible for the growth of head furnishings and rudimentary Wolffian ducts while

the female hormone liberated by the stimulated cortical tissue is responsible for the growth of the oviduct.

6480

Transient Hyperglycemia and Glycosuria Following Discontinuation of Insulin Given Non-Diabetic Patients.

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From the Laboratory of Pathological Chemistry and the Department of Internal Medicine, State University of Iowa.

The rise in blood sugar following the discontinuation of insulin administered to an anorexia case suggested that a temporary hyperglycemia and even a glycosuria might be induced in this way in non-diabetic subjects. Accordingly, 5 other non-diabetic patients were given insulin, starting with Units V before each meal, and the dosage increased at intervals of a few days until one patient was receiving Units XVII and another Units XXV 3 times a day. The injections were given 15 to 30 minutes before meals. Blood sugars 2 hr. after meals were usually normal, sometimes above normal. Hypoglycemic symptoms were rarely encountered. Upon discontinuation of the insulin all of these patients showed a hyperglycemia and 4 of them a temporary glycosuria. Glucose tolerance curves were done on 3 of the 6 patients and showed a diminished tolerance, even in one case where there was failure to produce a glycosuria.

For example, a female patient, age 34 years, entered the University Hospital June 2, 1932, following a pleurisy with effusion; she had lost weight and her appetite was poor. On June 4 a 50 gm. sugar tolerance test was normal, fasting blood sugar was 87 mg., the maximum at the half hour was 159 mg. Insulin was started at noon on this day with an initial dosage of Units V 3 times a day with the patient on a general diet. A second glucose tolerance test was done June 18 when the patient was receiving Units X 3 times a day; blood sugar reached a maximum of 231 mg. after 1 hr. The patient had been receiving insulin Units XXV 3 times a day on July 10 and insulin was discontinued the following day. A glucose tolerance test was of the mildly diabetic type, starting with a fasting figure of 87 mg. and with subsequent half-hour values of 208, 231, 253, and 176 mg.; the urine during the first hour contained 0.8% of sugar and during the second 1.1%. Tolerance

curves on July 16 and 19 showed that tolerance was slowly improving, but had not quite returned to normal 8 days after the insulin injections were stopped.

The hyperglycemia following cessation of the insulin injections in these non-diabetics might be explained as due to a compensatory inhibition of the normal islet secretion with a slow readjustment until ordinary activity is attained. Such an interpretation is in accord with the idea of a latent functional capability of the island cells in diabetes mellitus.[1] However, a study of the respiratory quotients in the insulinized non-diabetics now under way indicates that an explanation other than the above may obtain.

6481

On the Motion of Growth. IV. Further Analysis of Energetics of Heat Production with Special Reference to Basal Metabolism During Prolonged Human Fasting.

NORMAN C. WETZEL.

From the Babies and Childrens Hospital, Cleveland, and the Department of Pediatrics, School of Medicine, Western Reserve University.

Certain theoretical considerations in respect to the energetics of heat production as defined[1] for states of changing weight are of special interest but should also prove of rather great practical importance. In view of the fact that the hyperbolic term in equation (6) of the foregoing paper has dampened out and become negligible in the case of human growth beyond the age of twenty, this relation assumes the simpler form,

$$\rho\left(\frac{dq}{dt}\right)^2 + E_c\frac{dq}{dt} + A' = U \qquad (7)$$

for heat production in calories per Kg. per unit of time, the various factors possessing the same significance as before.

It is clear from (7) that U will not alter greatly during any period in which $\left(\frac{dq}{dt}\right)$ fails to change rapidly—a condition, as we have seen, which is actually reached on the average during the second and third decades of life. For constant values of $\pm\left(\frac{dq}{dt}\right)$, U

[1] Gibson, R. B., *J. Lab. Clin. Med.*, 1929, **14**, 597; PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 449; *Am. J. Physiol.*, 1932, **101**, 41.

[1] Wetzel, N. C., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 233.

will also remain constant; but an important question arises as to the conditions under which U, as representative of heat output, will be most economical. So long as $\left(\frac{dq}{dt}\right)$ is positive there cannot be a true minimum, although U may, of course, with low rates of reproduction become as small as ρ, E_c and A' will allow. If, however, no restriction be placed upon the sign of $\left(\frac{dq}{dt}\right)$, a rather significant result may be at once attained by differentiating (7) with respect to t and equating to 0, whence,

$$\frac{dq}{dt} = \frac{-E_c}{2\rho} \tag{8}$$

a condition which leads to the result that U, the unit rate of energy output in the form of heat will actually become and remain a minimum (for constant values of ρ, E_c and A') as long as the unit rate of change in weight $\left(\frac{dq}{dt}\right)$ is negative, and equal to $\left(\frac{-E_c}{2\rho}\right)$.

Attention is thus immediately directed to the possibilities of studying heat production during periods of starvation. But before giving our results, we ought briefly to continue the investigation of these equations. Substituting the value of $\left(\frac{dq}{dt}\right)$ from (8) into (7), we have,

$$A' - \frac{(E_c^2)_s}{4\rho_s} = [U_s]_{\text{minimum}} \tag{9}$$

wherein the subscript s now refers to starvation. The latter result thus indicates that a fasting subject who is losing weight at a unit rate, $\left(\frac{E_c}{2\rho}\right)_s$ must necessarily manifest a rate of heat production which will not only remain constant during this epoch, but which will actually be less than the "equilibrium" level of metabolism represented by A'. It is also of interest to see from the above set of equations that the value of $\left(\frac{dq}{dt}\right)$ from (8) which thus insures a minimum rate of metabolism, is exactly one-half as great as that which would allow a fasting subject likewise to maintain a constant rate of heat production at, however, precisely the "equilibrium" level A', for, if,

$$\frac{dq}{dt} = \left[\frac{-E_c}{\rho}\right]_s \tag{10}$$

we get from (7)

$$A' = U_s \tag{11}$$

The foregoing theoretical results thus afford a rather likely explanation of certain analogous experimental observations long ago

described for fasting animals by Rubner[2] and in later years confirmed by Benedict and coworkers[3, 4] in human and in animal (rat) starvation. They cannot be fully discussed here.

As further evidence of the present concept of energy exchange in respect to growth in general and in respect to the particular theoretical results just described, we present next the essential calculations in the human case based upon the excellent experimental data of Benedict.[4] To test (7) it is merely necessary to substitute values of $\left(\frac{dq}{dt}\right)$ which may be computed from data on weight during the period of starvation by any sufficiently exact method, as well as the observed values for U, and calculate the resulting numerical values:

$$\rho_s = 137.256$$
$$(E_c)_s = 641.260$$

when U is given in Cal./Kg./Year, and A' is taken as 9250 in the same units.* Resubstituting and computing "theoretical" values on successive days for U we obtain the final results arranged in Table I and shown graphically in Figure 1. The latter demonstrate conclusively that our assumptions and procedures are quite in accordance with observation.

TABLE I.
Theoretical Values for Basal Metabolism during the Course of a Human Fast Observed by Benedict.

Day of Fast	U_s in Cal./Kg./hr.		
	Computed from Equation (7)	Observed Bed Calorimeter	Observed by Indirect Calorimetry
0	——	1.12	1.05
1	1.163	1.07	1.13
2	1.121	1.09	1.11
3	1.079	1.10	1.10
4	1.046	1.10	1.05
5	1.013	1.02	1.03
6	0.989	1.04	1.01
7	0.974	1.02	1.04
8	0.971	1.06	1.04
9–31	0.970	0.9361 (mean)	0.9678 (mean)

[2] Rubner, Max, *Die Gesetze des Energieverbrauchs bei der Ernährung*, Leipzig, 1902.

[3] Horst, Kathyrn, Mendel, L. B., and Benedict, F. G., *J. Nutrition*, 1930, **3**, 177.

[4] Benedict, F. G., A Study of Prolonged Fasting, Carnegie Institution Publication No. 203, Washington, 1915.

* In this case $\left(\frac{dq}{dt}\right)$ is to be computed in Kg./Kg./Year.

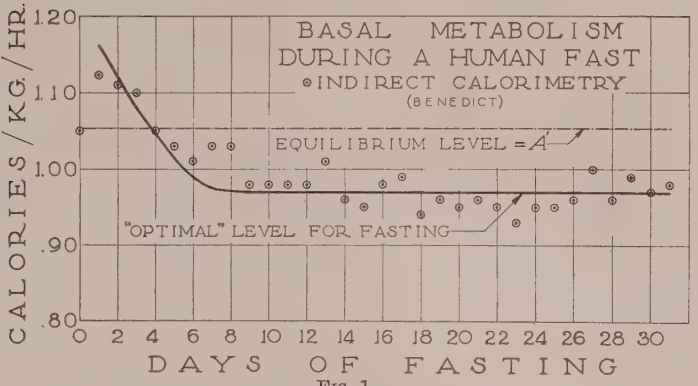

FIG. 1.

The smooth curve traces the theoretical course of heat production in Benedict's study of Levanzin as this has been computed from equation (7). Note that the curve descends below the equilibrium value A' and remains at its lowest level throughout the remainder of the fast, to fulfill the condition for "optimal" energy transfer.

To sum up we have shown that:

(1) The unit rate of energy evolution in heat, U, will be greater than that at the "equilibrium" level A' for all unit rates of change in weight greater than 0;

(2) For negative unit rates of changes in weight (starvation),

$$U = A' \quad \text{when} \quad \frac{-dq}{dt} = 0, \text{ or, } \frac{(E_c)_s}{\rho_s}$$

$$U \gtrless A' \quad \text{when} \quad \frac{-dq}{dt} \gtrless \frac{(E_c)_s}{\rho_s}$$

$$U \text{ is minimum when } \frac{-dq}{dt} = \frac{(E_c)_s}{2\rho_s}$$

The numerical results herein computed for $(E_c)_s$ and ρ lead finally to the prediction by way of equation (8) that the rate of loss in weight during human starvation which ought to insure a minimum rate of heat production is 2.336 Kg./Kg./Year or 0.0064 Kg./Kg./Day, a result next to be tested and confirmed in the following paper.

Some further implications of the equations thus far brought forward find excellent confirmation in the most recent observations of Benedict, Horst, and Mendel[5] on the basal metabolism of oversized starving rats. Their data for the rat again support in every

[5] Benedict, F. G., Horst, Kathryn, and Mendel, L. B., *J. Nutrition*, 1932, **5**, 581.

important respect the major theoretical features of both weight and metabolism curves of human fasting subjects upon which we have laid special emphasis in this and in the succeeding paper. The experiments show clearly (1) that oversized rats also pass into a period of almost purely logarithmic loss in weight (optimal stage where $\left(\frac{dq}{dt}\right) = \text{constant}$; (2) that basal metabolism in terms of Cal./Kg./Day recedes *below* the equilibrium level and remains *constant* during this phase; and (3) as we must expect from equation (7) a subsequent *increase* in U when $\left(\frac{dq}{dt}\right)$ rises at a later stage of the process, in virtue of the absence of the original source of energy S in equation (1).[6] The latter increase in the velocity of starvation will be discussed more extensively in another paper;[7] but, the rise of basal heat production, U, under such conditions is a direct outcome of the energetics of metabolism set forth in equation (7).

6482

On the Motion of Growth. V. Rate of Loss in Weight for Minimum Metabolism.

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From the Babies and Childrens Hospital, Cleveland, and the Department of Pediatrics, School of Medicine, Western Reserve University.

From theoretical considerations in conjunction with data on heat production during prolonged fasting it has been shown[1] that the optimal relative rate of starvation at which a human individual would conserve his body reserves to the utmost ought to be closely in the neighborhood of 0.0064 Kg./Kg./Day. It is now proposed to examine this result in the light of several observations on the point.

By far the most accurate of the data at hand are those reported by Benedict,[2] but in Figure 1 we have also included the curves of Succi for several fasts, as well as that for Beauté. The ordinates are in $\log_{10}$ (Weight) $= .4343q$, the abscissae in days. There is,

[6] Wetzel, N. C., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 224.

[7] Wetzel, N. C., to be published.

[1] Wetzel, N. C., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 354.

[2] Benedict, F. G., A Study of Prolonged Fasting, Carnegie Institution Publication No. 203, Washington, 1915.

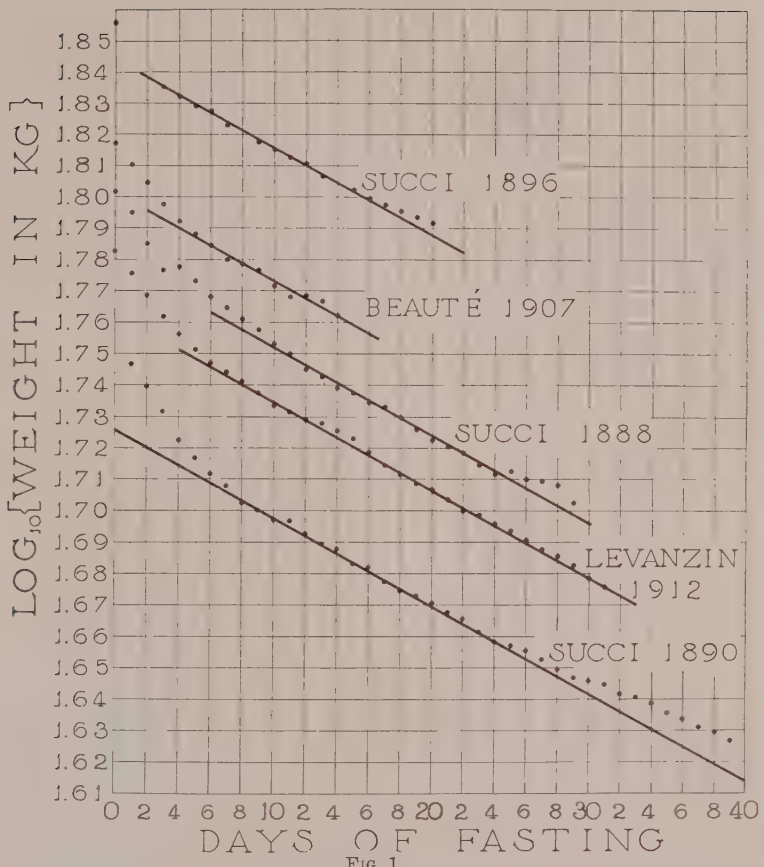

FIG. 1.

The parallel lines drawn through the several sets of observations indicate the expected slope of 0.0064 Kg./Kg./Day during the apparently linear phase of loss in weight.

here, a striking similarity not only in the almost identical shape of these curves, but also in their slope, especially after the 4th or 5th day of fasting. This slope appears so decidedly linear that purely graphical analysis could hardly detect any significant curvature. We shall call this the "apparently linear" phase.

Taking the data on Levanzin by Benedict for each day from the 8th to the 30th inclusively and analyzing this apparently linear phase by least squares, we find that the slope is 0.00645 Kg./Kg./Day, a numerical result completely confirming that given above and already reached by an independent method previously described.[1]

Straight lines have also been drawn through the other examples

and indicate the expected slope, 0.0064. These likewise pass excellently through their respective points in every case, notwithstanding the diverse conditions and times of observation.

But, even though the foregoing value for an "optimal" rate of starvation is actually witnessed in practice—we must avoid the conclusion that starvation would necessarily continue indefinitely in this way. Indeed, as it will appear in a subsequent paper, the "linear" phase in such experiments discloses merely a temporary, though an admirably close approach to what are fundamentally "ideal" conditions of starvation for the individual subject and for the kinetic system his organism represents. The true nature of the starvation curve is decidedly more complex, and will be described elsewhere.

6483

On the Motion of Growth. VI. Energetics of Bacterial Growth and Heat Production.

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From the Babies and Childrens Hospital, Cleveland, and the Department of Pediatrics, School of Medicine, Western Reserve University.

In several previous communications[1-5] we have outlined a concept in which the phenomena of growth and metabolism are viewed as separate forms of a single underlying mode of motion. A scaffolding is thus provided by which each of these major processes may be studied more directively. But our descriptions up to this point have been restricted to the special case of human growth and metabolism, and it is therefore proposed, since the methods and the theory are sufficiently general, to consider an example of the cognate phenomena in a population of unicellular organisms. In order, however, to apply the present theory, it is essential that we possess simultaneous information upon the two chief processes in action, namely, growth and coincidental heat production. Such data, for our purposes, need to be more than ordinarily accurate, else analysis is labor in vain. Considering the difficulties at hand, there is small wonder that suitable observations are few; indeed, in the bacteriological field there are no data which are more worthy of attention and study than those reported within the past few years by

1-5 Wetzel, N. C., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 224, 227, 233, 354, 358.

Bayne-Jones and Rhees.[6] We shall employ the data on growth and simultaneous heat production as recorded for their experiment 2 in order to illustrate the general method of application of our own results.

The Growth Curves.

The equations of motion are essentially the same as those already given in the case of human growth, save for the fact that the right hand side of,

$$\lambda\frac{d^2q}{dt^2}+\rho\frac{dq}{dt}+\frac{q}{\kappa}=E \tag{12}$$

namely, the "applied force" representing $(E_s—E_c)$, the difference between the potential of energy at the source and cell respectively, is sufficiently constant to be treated so in this as well as in all other examples of bacterial growth which we have thus far investigated. But, in view of the fact that the descriminant,

$$\left[\left(\frac{\rho}{\lambda}\right)^2-\frac{4}{\lambda\kappa}\right]<0 \tag{13}$$

in these instances, the solution of (12) may take the form,

$$q=E\kappa+K\epsilon^{-\alpha t}\sin(\beta t+\theta) \tag{14}$$

α representing the real, β the imaginary part of the roots of (12); K and θ being arbitrary for integration.

From the latter equation we should expect the curves of bacterial growth to exhibit real oscillatory deflections that are dampened by the factor α which, like β contains the fundamental constants of growth and metabolism, λ, ρ, and κ, implicitly. Applying (14) to the data mentioned, we get, by methods later to be described:[7]

$E\kappa = 10.734$ $\qquad$ $K = —5.94405$
$\alpha = 0.6544$ $\qquad$ $\theta = 0.302346$
$\beta = 0.187$

when q is transformed to the Briggsian base. The curve of (14) computed with the aid of the foregoing values is illustrated in Figure 1. The numerical results are arranged in Table I. Graphical adjustment is obviously satisfactory, and shows, contrary to the general opinion frequently expressed, that the algebraic representation of bacterial growth during all of the well-known phases of reduplication is quite within the range of analysis.

[6] Bayne-Jones, S., and Rhees, Henrietta, *J. Bact.*, 1929, **17**, 123.

[7] Wetzel, N. C., to be published.

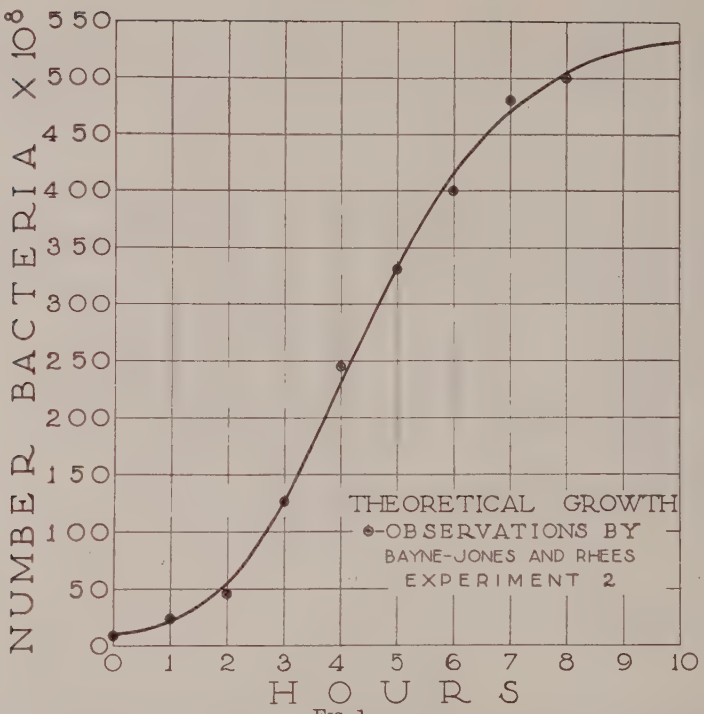

FIG. 1.

The smooth curve computed by means of equation (14) traces the theoretical course of growth in the culture observed by Bayne-Jones and Rhees,[6] and meets the data satisfactorily.

TABLE I.

A Comparison of the Theoretical and Observed Values for Cell Number and Total Heat Production in Experiment (2) of Bayne-Jones and Rhees.

Age of Culture	Number of Bacteria (*z*)		Total Heat, $\mathfrak{T}_p$	
	Observed	Calculated from Equation (14)	Observed	Calculated from Equation (17)
hrs.	$\times 10^8$	$\times 10^8$	*gm. cal.*	*gm. cal.*
0	8.5	9.2	——	——
1	20.5	19.4	1.19	——
2	45.4	53.4	8.95	9.52
3	126.0	126.0	16.30	15.10
4	245.0	229.0	20.90	21.27
5	330.0	332.0	25.90	26.58
6	400.0	415.0	31.00	31.56
7	480.0	470.0	36.10	36.10
8	500.0	505.0	40.50	40.54
9	——	524.0	45.20	45.05
10	——	533.0	49.90	49.91

Heat Production.

As before[8] we turn to,

$$\rho\left(\frac{dq}{dt}\right)^2 + E_a\frac{dq}{dt} + A' = U \tag{15}$$

for the theoretical course of unit rate of heat production. Since, however, we have found that E_a is small as compared with ρ in the present case, equation (15) may be reduced to,

$$\rho\left(\frac{dq}{dt}\right)^2 + A' = U_a \tag{16}$$

from which the unit rate of heat production, U_a is now composed simply of heat dissipated and heat returned from maintenance.

The evaluation of ρ and A' could easily be completed were it possible reasonably to approximate the correct values of U_a (gm. cal./cell/hr.) from the original observations which happen, however, to have been made in such a manner that total cumulative heat, and not rate of heat production was measured in the calorimeter. Hence, to make comparisons most satisfactory, we need here to rearrange and to proceed somewhat differently with (16). The foregoing equation is therefore multiplied by z (cell number) as given in terms of t with the aid of (14), remembering that $z = \epsilon^q$, and integrated once with respect to the independent variable. The result symbolically,

$$\rho\int z\left(\frac{dq}{dt}\right)^2 dt + A'\int z\,dt = \mathfrak{U}_\rho + C_o \tag{17}$$

must, unfortunately, be partly expressed in series form, at least six terms of which will be required for computing $\mathfrak{U}_\rho$, the total heat produced by z organisms up to and including time t. With the integrals in (17) evaluated and with $\mathfrak{U}_\rho$ given directly by the data, we are finally able to solve a set of equations of this type simultaneously for ρ, A', and C_o. We have thus found,

$$\rho = 0.0007652 \times 10^{-6}$$
$$A' = 0.00009 \times 10^{-6} \text{ gm. cal./cell/hr.}$$
$$C_o = -25.7, \text{ the constant of integration}$$

The details of the foregoing procedures, as illustrated in another example of similar nature, are being published elsewhere.[9]

By resubstituting the results just given into (17), hourly theoretical values for total cumulative heat, $\mathfrak{U}_\rho$, have been computed*

[8] Wetzel, N. C., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 233.

* With the aid of the corresponding values of $\left(\frac{dq}{dt}\right)$ as obtained from the first derivative of (14).

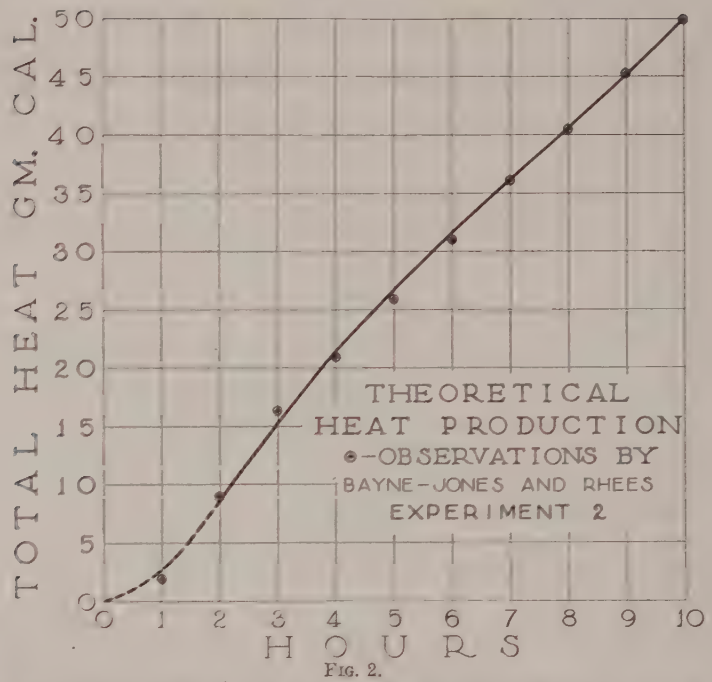

FIG. 2.

The theoretical course of heat production in the same experiment as in Fig. 1. The smooth portion of the curve has been computed from the integral of (17); the earlier portion by mechanical means from the curve of (16).

and are given in Table I. They trace the curve in Fig. 2. The correspondence is about all that could be expected in view of both experimental as well as analytical difficulties necessarily encountered. Consequently, it is worth emphasizing that the theoretical relations (15) and (16) which are thus seen to redescribe the course of bacterial heat production, just as they have succeeded in doing this for the human case, are actually part and parcel of the fundamental equations of growth set forth in this series of papers. No full discussion can be entered into here, although it is evident that the implications of these results are sufficient to warrant the application of present methods to further observations of a similar nature. In fact, as we shall later show,[9, 10] there is no other method, once p has been determined, by which the unit *rate* of heat production can be as accurately computed for any and all phases of growth as by equation (15) or even (16). Such information is vitally

[9] Wetzel, N. C., to be published.

[10] Wetzel, N. C., to be published.

important to studies of bacterial growth and metabolism, but beyond this, there is at present, no other way by which the numerical values of the fundamental growth constants, λ, ρ, and κ can be determined, and consequently, no other means of approach to a further study of their ultimate physical and physiological properties.

6484

Influence of Raw and Whole Dried Liver on Food Consumption and Utilization.*

WALTER H. SEEGERS AND H. GREGG SMITH. (Introduced by H. A. Mattill.)

From the Biochemical Laboratories, State University of Iowa, Iowa City.

In experiments described by Smith[1] it was shown that liver tissue extracted with alcohol until fat-free has a biological value inferior to that of whole liver, as shown by a subnormal growth rate. In the present experiments an attempt was made to gain information as to the manner in which whole liver exerts its favorable influence. A combination of the efficiency quotient method of Palmer and Kennedy[2] and the paired-feeding method used by Mitchell and Carman[3] was used. Male albino rats on a complete diet containing 20% extracted liver,[1] supplemented by 0.5 gm. of dried whole liver or 1.5 gm. of raw liver daily, and whose food intake was limited to that of control animals, grew at a faster rate and had lower efficiency quotients than did the controls. Animals receiving the supplements

TABLE I.

Exp. No.	Liver Supplement	Prelim. Period 21 days Food	Prelim. Period 21 days E.Q.	Exp. Period 40 days Wt. Start	Exp. Period 40 days Gain Wt.	Exp. Period 40 days E.Q.	Exp. Period 40 days Food	Remarks
		gm.		gm.	gm.		gm.	
1	—	126	4.66	74	100	2.53	314	Control
	Whole	105	4.42	73	115	2.10	314	Limited
	"	118	4.63	80	146	1.82	416	Unrestricted
2	—	171	4.38	101	123	2.25	449	Control
	Ext'd	111	4.33	76	120	2.69	439	Limited
	"	120	4.33	88	139	2.10	459	Unrestricted

* This work was in part supported by a grant from the National Live Stock and Meat Board.

[1] Smith, H. G., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 597; 1932, **29**, 669.

[2] Palmer, L. S., and Kennedy, C., *J. Biol. Chem.*, 1931, **90**, 545.

[3] Mitchell, H. H., and Carman, C. G., *J. Biol. Chem.*, 1926, **68**, 165.

and having no restrictions in food intake consumed more food, but made greater gains and had somewhat lower efficiency quotients than those whose food intake was limited. When the diet was supplemented with an equivalent amount of alcohol-extracted liver (0.5 gm.), the animals with limited and with unrestricted food consumption made no greater, or but slightly greater, gains than did the control animals and also had similar efficiency quotients. In all, 8 experiments (24 animals) with the whole liver and 4 (12 animals) with the extracted-liver supplements were carried out. A typical experiment with each of the supplements is shown in Table I.

It appears, therefore, that the increased growth rate resulting from feeding whole liver is due to some influence other than an increased food consumption, and that the factor responsible for the growth-promoting effect is removed by alcohol.

6485

Respiratory Quotient of Various Parts of the Brain

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The respiratory quotients of minced brain stem, cerebellum and medulla of fed rats were studied in the Warburg apparatus. The tissues were suspended in Ringer solution containing 0.1% glucose and buffered with phosphate at pH 7.4. The results are presented in Table 1.

Further work is in progress in an attempt to determine the ability of the brain to oxidize various food stuffs.

TABLE I.

Part	No. Observations	Average RQ	Deviation of Mean
Cortex	8	0.99	±0.001
Brain stem	30	0.93	±0.001
Cerebellum	25	0.89	±0.002
Medulla	25	0.89	±0.002

6486

Effect of Viosterol on Excretion of Lead.*

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It is well known that large doses of viosterol cause a hypercalcemia and increased elimination of calcium from the body and good evidence is accumulating to the effect that the source of the excess of the calcium under such conditions is from the bone, at least when the diet is calcium poor.[1, 2, 3] Since Aub[4] has shown that the metabolism of lead parallels the metabolism of calcium, large doses of viosterol should promote the elimination of lead from the body. Experiments to determine the effect of large doses of viosterol on lead excretion in cats and guinea pigs and on lead storage, and the course of lead poisoning in guinea pigs were carried out. Throughout the series Viosterol Squibb 250D was used. All assays for lead were made by the Fairhall method. The guinea pigs were kept on a normal diet of oats, hay, cabbage, or lettuce. The cats were given low calcium diets consisting of meat and fish or vegetables. Viosterol dosage in the 2 cat experiments was 20 drops daily and in the case of the guinea pigs, 6 drops daily in 2 experiments and 10 drops daily in one.

In both cat experiments the excretion of lead was immediately increased after the administration of viosterol. In the first experiment the viosterol cats excreted more lead than the control group in each of 5 three-day periods. The difference between the groups was greatest in the second period when the viosterol cats showed a lead elimination 10 times greater than the control group.

In the second cat experiment the average daily excretion of lead per cat rose sharply in the first 3 day period after viosterol was begun and reached a peak in the fourth period. At the peak the amount of lead excreted per cat was 37 times greater than it had been in the control period. In the case of the guinea pigs, lead

* The work on which this paper is based was done on a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

1 Taylor and Weld, *Am. J. Physiol.*, 1931, **97**, 566.

2 Brown and Shohl, *J. Biol. Chem.*, 1930, **86**, 245.

3 Hess, Benjamin and Gross, *J. Biol. Chem.*, 1931, **94**, 1.

4 Aub, Fairhall, Minot, Reznikoff, Lead Poisoning, 1926.

elimination after viosterol increased very slightly as compared with its increase in the cat experiments. This may possibly be accounted for by the relative resistance of guinea pigs to viosterol spoken of by Taylor and his associates.[5]

An experiment in which viosterol was given to 4 guinea pigs for one week prior to, and during lead administration, was undertaken to determine primarily whether the viosterol would have any protective action against lead poisoning. It was thought that by promoting rapid elimination the danger of poisoning might be lessened. Although the number of animals was small the result of the experiment seemed to indicate quite definitely the opposite of any protective effect; death occurring in all 4 viosterol pigs more promptly than in the case of 10 out of 11 controls.

A comparison was made between the amounts of lead retained in the bodies of guinea pigs which had been given viosterol before and during lead administration and the lead in the bodies of pigs which had received lead alone. Two viosterol pigs showed a considerably greater retention of lead in their bodies after death than was shown by the controls and 2 showed less.

6487

Absorption Rates of Galactose and Mannose.*

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The absorption rates of galactose and mannose were studied in rats and dogs using one hour as the absorption period. The rats were not fed during the 48 hours before being used but water was given as usual. One gram (2 cc. of a 50% sol.) of the sugar was given by stomach tube. Animals were killed by a blow on the head after which a ligature was tightly tied about the neck to prevent loss of sugar by mouth. The abdomen was then opened and ligatures placed about the oesophagus and lower end of ileum. This portion of the gastrointestinal tract was removed and the unabsorbed sugar washed out with warm water for quantity determinations.

[5] Taylor, Weld, Branion and Kay, *Can. Med. Assn. J.*, 1931, **24**, 763.

* Aided by Grant 222 from the Committee on Scientific Research, American Medical Association.

In using closed loops of ileum in dogs 2 gm. of the sugar were given (10 cc. of a 20% sol. introduced by needle and syringe). At the end of an hour the content was aspirated and the loop washed by injecting warm physiological salt solution and aspirating. More complete description of these procedures has been published.[1]

Sugars of definite rotation (d-galactose + 80.5°, d-mannose + 14.25°) were used and concentrations made by weight were checked by polariscope. Galactose determinations were made by the Shaffer-Hartman method for determining glucose, consequently 10% had to be added to the findings to approximate the actual amount of galactose present. This method can not be used with any degree of accuracy for mannose estimations. A satisfactory method consists in reducing Benedict's qualitative solution and then plating the copper out and weighing the copper electrodes.[2]

The results are shown in Table 1. While there is some difference in the comparative rates at which these 2 sugars were taken up by dogs and rats, the findings would seem to justify the conclusion that galactose is absorbed approximately twice as fast as mannose.

Using similar methods we have reported the relative rates of absorption of d-glucose and d-fructose.[1] Cori[3] investigated the

TABLE I.

Absorption of galactose and mannose in gms. in different animals. 10 cc. of 20% solution of sugar used in the dog closed loops. 2 cc. of 50% sugar solution in the rat alimentary tracts. Time of absorption, one hour. Rat weight limits 140-240 gm. Average 168 gm.

Closed Loops (Dogs)		Gastrointestinal Tracts (Rats)		
Galactose	Mannose	Galactose	Mannose	
1.02	.45	.69	.33	.40
0.91	.56	.64	.35	.35
0.93	.43	.55	.40	.24
0.83	.57	.78	.38	.47
0.91	.30	.68	.37	.27
0.83	.36	.70	.25	.22
0.72	.43	.61	.33	.46
1.10	.36	.54	.30	.48
1.06	.56	.59	.28	.45
1.00	.24	.67	.36	.30
0.84	.31	.47	.43	.23
0.88	.32	.63	.45	.26
0.95	.43		.44	
0.88	.56		.37	
1.02			.28	
0.93			.38	
0.93			.40	
Average 0.93	.42	.63		.35

[1] Burget, G. E., Moore, P., and Lloyd, R., *Am. J. Physiol.*, 1932, **101**, 565.

[2] Moore, P., Lloyd, R., and Burget, G. E., *J. Biol. Chem.*, 1932, **97**, 345.

[3] Cori, C. F., *J. Biol. Chem.*, 1925, **66**, 691.

TABLE II.
Comparison of the rates of absorption of hexoses (glucose = 100).
Absorption time, 1 hr.

Sugar	Rats	Closed loops (dogs)
d-galactose	115	108
d-glucose	100	100
d-fructose	91	92
d-mannose	64	49

rates of absorption of these 4 sugars in rats. On the basis that glucose = 100, he states the absorption rate of galactose as 110, fructose 43 and mannose 19. On this basis (glucose = 100) our findings are summarized in Table 2. There are probably 2 factors that enter into this difference of results, namely, the number of experiments necessary in this type of work to allow one to arrive at a dependable average and the method necessary to accurately estimate small quantities of mannose.[2] While the Shaffer-Hartman method for glucose determinations may be applied to levulose and galactose since the error remains fairly uniform throughout the range of concentrations used, no uniformity was found when applying it to mannose determinations.

The marked difference in reaction of glucose and mannose to a common reagent emphasizes the importance of their stereoisomerism and at once suggests this as the probable factor in the difference in absorption rates rather than the so-called "selectivity" (preference for glucose) on the part of the intestinal mucosa.

6488

The Head Pattern in Amblystoma Studied by Vital Staining and Transplantation Methods.

ESTHER CARPENTER. (Introduced by Ross G. Harrison.)

From the Osborn Zoological Laboratories, Yale University.

Local vital stains were applied to the prospective head region in the late yolk plug stage of *Amblystoma punctatum* by Vogt's[1] method. From the records obtained by making camera lucida drawings of the position and extent of the stained areas in the stages following, composite diagrams were constructed showing the arrangement of the parts of the prospective ectoderm involved in the

[1] Vogt, *Arch. f. Ent. d. Org.*, 1925, 106.

formation of the nose, lens, ear, balancer, gills and stomodaeum. Two of these, Harrison stages 13 (slit blastopore) and 22 (shortly after closure of neural folds), are shown in the figures.

The wheeling about of the head epidermis due to the dorsal convergence of the neural material which lies almost out at the equator of the embryo in the gastrula stage, takes place in *Amblystoma punctatum* in a manner similar to that observed by Vogt[2] and Goerttler[3] on European salamanders. There is a forward and downward growth of the ectoderm of the head region anteriorly in such a way that the material at the middle of the transverse fold comes to lie just in front of the stomodaeum and ventral to the eye—a shifting of the material nearest the dorsal midline in an anterior and ventral direction while that originally ventral moves caudally and dorsally.

The results obtained for Amblystoma are much less diagrammatic than those Röhlich[4] gives for Triton but the arrangement of the anlagen is essentially the same in both forms. The position of the lens agrees with that given by both Manchot[5] and Röhlich for Triton. In Amblystoma however the nasal ectoderm seems to extend up on the outer side of the neural fold (stage 15) for a short distance and the ear ectoderm not quite to reach the fold. The latter may also be slightly farther anterior in Amblystoma than in Triton.

On the basis of the vital staining results transplantation experiments have been undertaken, using stained and unstained embryos of *Amblystoma punctatum* of the same age (donor and host) to test the relative determination of the balancer, lens and nasal ectoderm during the neurula stages. The results so far obtained indicate that nasal ectoderm is determined earlier than either balancer or lens as shown by the test of self-differentiation when transplanted to other parts of the head region. This capacity was evident in nasal ectoderm from stage 14 (just before appearance of medullary folds) on. Nasal placodes developed in the balancer region, between the balancer and the eye, in the mouth region and on the surface of the first gill. From stage 14 on replacement of part of the nasal ectoderm by other head ectoderm brought about a reduction in size and retardation in development of the nasal placode. The lens ectoderm becomes a segregate in Lillie's[6] sense of the word

[2] Vogt. *Arch. f. Ent. d. Org.*, 1929, 120.
[3] Goerttler, *Arch. f. Ent. d. Org.*, 1925, 106.
[4] Röhlich, *Arch. f. Ent. d. Org.*, 1931, 124.
[5] Manchot, *Arch. f. Ent. d. Org.*, 1929, 116.
[6] Lillie, *Arch. f. Ent. d. Org.*, 1929, 118.

later than either the nose or balancer. Lentoids, i. e. partially differentiated lenses, were the usual result of transplantation of lens ectoderm to other parts of the head region even as late as stage 20 (contact of neural folds), although one case of apparent self-differentiation into a vesicular lens was obtained as early as stage 14. Lenses normal in state of differentiation but decreasing in size with the increase in age at which the operation was performed were obtained when lens ectoderm was completely replaced between stages

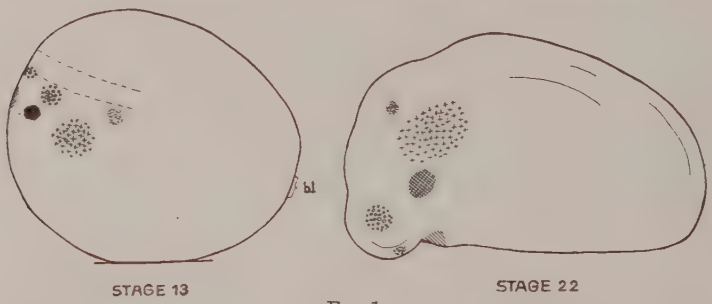

FIG. 1.

The areas of the ectoderm indicated are as follows: Large stippling—nasal; open circles—lens; small stippling—ear; slanting lines—stomodaeum; cross-hatching—balancer; crosses—gill mound; broken lines—medullary fold. The position of the blastopore is indicated by the letters—bl.

14 and 20. Up to stage 19 as much as 2/3 of the lens ectoderm can be replaced by other head ectoderm without interfering with the development of a lens normal in size and stage of differentiation. In one case suppression of the balancer was effected by covering the region with other head ectoderm at stage 13 + (before the appearance of the medullary folds). Sections made after the balancer on the unoperated side was well developed showed a thickening of the basement membrane on the opposite side and an increase in the thickness of the lower layer of the ectoderm similar to that observed during the early development of the balancer.[7] No cases have yet been obtained of self-differentiation of balancer ectoderm at stages before the formation of the medullary folds.

[7] *J. Exp. Zool.*, 1925, **41**.

6489

The Force-Velocity Curve of Striated Muscle.

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When the force which is exerted by a muscle is plotted, on the ordinates, against the speed with which it shortens, on the abscissae, the curve relating the two variables is, in general, a straight line sloping towards the x axis. This relationship between force and velocity means that the force exerted by a muscle diminishes as its speed of contraction increases. This result has appeared in the work of Gasser and Hill,[1] Fenn[2] and in our own experiments soon to be published at length elsewhere. The purpose of this paper is to report a fact which emerges from our data which seems to throw light on the cause of the force-velocity relation.

Our experiments were made on the gastrocnemius muscle of the decerebrate cat stimulated through the tibial nerve by a single maximal, break, shock of a Harvard inductorium. The muscular response was, therefore, a twitch. The muscle contracted against an inertia disc (radius 8.65 cm.) the moment of inertia of which was 290,400 gm.-cm.^{2} The displacement of the disc was recorded optically. Time was imprinted on the record in intervals of .001″ by means of a slotted disc which was rotated by a synchronous motor in such a way as to interrupt a thin beam of light from a 500 watt lamp. The displacement of the inertia disc was measured from the photographic record with a cathetometer for each .001″ and from these measurements, by taking first and second differences, the angular velocity and angular acceleration of the disc were computed. Although this record was measured to each .001″, we have used in this computation, the interval of .01″. The force, calculated from the moment of inertia and the angular acceleration of the disc, is, therefore, known for each time interval (.01″) during the contraction cycle. The shortening of the muscle was also simultaneously and independently recorded on the same record by means of a fine steel wire which drew, in a straight line without magnification an aluminum pointer in front of the camera as the muscle contracted. The change of length of the muscle for each .01″ was measured in the same manner as that described above for the measurement of the

[1] Gasser and Hill, *Proc. Roy. Soc., B.*, 1924, **96**, 398.

[2] Fenn, Brody and Petrilli, *Am. J. Physiol.*, 1931, **97**, 1.

angular velocity of the inertia disc. From the measurement of the change of length of the muscle for each .01″ the linear speed of contraction of the muscle for the same time interval was obtained. The force of the muscle, the speed with which it contracts and the shortening of the muscle in cm. are, therefore, known for each .01″. From these basic data, the work and power of the muscle for each time interval have been computed.

In Table 1, are presented the results of a single experiment on Cat 474 Record 3 which is typical of the twitch in a series of 20 animals. This animal was a pregnant female which weighed 2.8 kg. The length of the muscle was 9.5 cm. under an initial tension of 226 gm. Column 1 gives the time intervals in .005″ in order to allow the data to be arranged in the table as they should be plotted on the time scale. Velocities are placed on the half hundredths because the values are the average velocities over the whole period of .01″. Columns 2 and 3 give the muscle shortening in centimeters and also the change of length for each .01″. Column 4 gives the linear muscle velocity in cm/sec. Column 5 gives the angular velocity of the disc in radians/sec. Column 6 gives the angular acceleration of the disc in radians/sec.^{2} and column 7 the force in kg. Force in dynes is computed by multiplying the moment of inertia of the disc by the acceleration and dividing this product by 8.65 cm., the radius of the disc. Force in dynes is converted into force in kg. by dividing the former by 980,000. The term power is used in its technical signification which means the rate at which work is done. Power is equal to the work done in unit time divided by the same time interval and it is also equal to the force exerted in unit time multiplied by the corresponding muscle velocity. Power, Column 9, is the product of force, Column 7, and muscle velocity, Column 4. The figure for the muscle velocity is obtained by taking the average of 2 adjacent values to find the muscle velocity which corresponds in time to the force in Column 7. This figure is the figure in brackets in Column 4. Work, Column 8, is obtained by multiplying the figure for power by .01″.

The fact brought out by these experiments, which is significant for the interpretation of the force-velocity curve is the approximate constancy of power (Column 9) after the muscle has attained its maximal force. Power = Force x Velocity = a Constant. It follows from this equation that force and velocity vary inversely. Therefore, if velocity increases, force must necessarily diminish. The inference, therefore, appears to be warranted that in the work cited above, the force-velocity relation obtains because muscles move

during a part of their cycle under constant power. In none of the work hitherto published was the speed of the muscle independently and simultaneously recorded so that it has not been possible to compute from that data the power of the muscle from moment to moment during contraction.

It may be pointed out that the fact that muscle contracts under approximately constant power, after maximal tension has been developed, lends some weight to the theory that there are 2 sources of energy in a contracting muscle: an electro-chemical source which contributes the sudden development of maximal tension within the first 30 sigma after stimulation and an elastic source which absorbs the initial outburst of energy and delivers it at such a rate that the muscle does its work under constant power.

TABLE I.

1 Time in .005″	2 Muscle Shortening, cm.	3 Change of Length of Muscle per .01″, cm.	4 Muscle Velocity cm./sec.	5 Angular Velocity of Disc rad/sec.	6 Angular Acceleration of disc rad/sec.^{2}	7 Force, kg.	8 Work, kg./cm.	9 Power=f v
0	0	0	0	0	0	0	0	0
.0025″			[2]		70.0	2.400	.048	4.80
.005″			4	0.34				
.010″	.04	.04	[7.5]		93.0	3.180	.238	23.82
.015″			11	1.27				
.020″	.15	.11	[13]		46.0	1.580	.205	20.54
.025″			15	1.735				
.030″	.30	.15	[15]		34.5	1.180	.177	17.70
.035″			15	2.08				
.040″	.45	.15	[15.5]		35.0	1.199	.185	18.58
.045″			16	2.43				
.050″	.61	.16			00.0			
.055″			12	2.43				
.060″	.73	.12						

6490

Relation Between Vitamin A Potency and Carotene Content of Green Plant Tissue.*

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The finding that carotene is provitamin A for certain species raises the question as to whether a direct determination of the carotene content of plant tissue, coupled with information with regard to the vitamin A potency of isolated carotene, could be substituted for the extended biological assay. Euler, Demole, Karrer and Walker[1] report that a carotene determination in plant material discloses the magnitude of its vitamin A activity.

For the determination of carotene a colorimetric method was used, based on the original procedure of Willstatter and Stoll[2] but modified after critical study by one of us.[3] The vitamin A technique was essentially that employed by Sherman and has been referred to in an earlier publication.[4]

Alfalfa samples 45, 46 and 49 were taken from adjacent parts of the same field. Nos. 45 and 46 were dried in a mechanical drier by artificial heat within a few hours after cutting and No. 49 was sun-dried in the field. Samples 61 and 62 were artificially-dried and sun-dried, respectively, but from different parts of the country. A stock solution of carotene from carrots was prepared by dissolving a weighed amount of the crystalline substance (M.P. 169-171°, corr.) in ethyl laurate and olive oil. A dilution was made with olive oil every 2 weeks and in order to insure a constant level of carotene intake a colorimetric determination was made before each dilution. Hydroquinone was added to all solutions to the extent of 1 mg. per cc. as an antioxidant.

Table 1 displays the feeding levels which are in a comparable range as to rat growth response. The data, which are presented only in part, reveal that on the basis of carotene alone a smaller

* Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Agricultural Biochemistry.

[1] Euler, H., Demole, V., Karrer, P., and Walker, O., *Helv. Chim. Acta*, 1930, **8**, 1078.

[2] Willstätter, R., and Stoll, A., *Untersuchungen über Chlorophyll*, Berlin, 1913.

[3] Chichester, D. F., Master's Thesis, Rutgers University, 1932.

[4] Russell, W. C., *J. Biol. Chem.*, 1929, **85**, 289.

TABLE I.

Sample	No.	Carotene content dry basis	Wt. daily supplement	Carotene in supplement as fed	Positive survivors†	Av. gain in body wt. 5-wk. test per.	No. animals
		%	mg.	γ(=0.001 mg.)	%	gm.	
Alfalfa	45	0.0037	21	0.71	89	14	9
"	46	0.0041	24	0.90	89	20	9
"	61	0.0059	15	0.83	90	18	10
"	62	0.0023	25	0.53	86	20	7
"	49	0.00017	150	0.23	73	15	11
Carotene	C_1			1.50	88	24	8
Alfalfa	61	0.0059	25	1.38	100	45	8

† Animals alive at 5 weeks and which had gained in weight.

amount of this substance in plant tissue is necessary to produce a given growth response in the rat than when isolated carotene is fed. This difference might be due to a greater potency of carotene in association with plant tissue, to the presence of some unknown substance which has growth-promoting properties in vitamin A deficient animals or to the conversion of part of the carotene in the plant tissue to vitamin A during the curing process. Thus the carotene contents of the supplements of the sun-dried samples Nos. 49 and 62, are less than those of the artificially dried, yet the animal response is of the same order. Furthermore, 1.5 international vitamin A units, (1.5γ), fed as carotene, caused an average growth response of 24 gm., yet practically the same number of units in a 25 mg. supplement of No. 61 resulted in an average response of 45 gm. There is the possibility that the method of analysis did not account for all of the carotene present, yet this is not likely in view of the critical study made of the procedure prior to the animal feeding trials.

Hence, while the carotene content of dried green plant tissue, determined colorimetrically, is a rough indication of vitamin A potency, the present data indicate that this determination cannot be substituted for the biological assay, and that the drying process may affect the relation of the carotene content to the animal response.

6491

Pharmacology of Some Saligenin Derivatives.

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One of the authors reported the remarkable antispasmodic properties of benzyl benzoate and of the benzyl esters for smooth muscle,[1] and the local anesthetic properties of benzyl alcohol.[2] Numerous attempts were made by different investigators to prepare and study analogous benzyl esters and derivatives of benzyl alcohol, with the object of improving the therapeutic value of the original substances. Thus, Hirschfelder[3] pointed out that hydroxy-benzyl alcohol, or saligenin, also produced local anesthesia and was water-soluble. While phenmethylol and saligenin were both potent local anesthetics they had also a mildly relaxant or anti-spasmodic effect on smooth muscle preparations. Starting with saligenin, we synthesized a series of halogenated and other derivatives, and studied them especially as to their local anesthetic properties and their anti-spasmodic effects on smooth muscle.

The following compounds were prepared: amido saligenin, nitro saligenin, nitroso saligenin, mono-chlor saligenin, di-chlor saligenin, mono-brom saligenin, di-brom saligenin, mono-iodo saligenin, di-iodo saligenin, chlor-brom saligenin, brom-iodo saligenin, brom-nitro saligenin, nitro saligenin benzoate and saligenin nitro benzoate. All these compounds are solids and the majority are soluble in water only to a limited extent. Di-iodo saligenin is a very poorly soluble substance, saturated solutions of which give concentrations of only 1 : 20,000.

The local anesthetic effects of these compounds were studied on sensory nerve endings of frogs' legs, on the conjunctivæ of cats and rabbits and by the wheal method in guinea pigs. Minimal concentrations of the drugs, as well as the duration of the anesthesia, were taken into consideration when comparing the relative efficiency of the different compounds. Beginning with the most potent, the relative local anesthesia produced by the series, in concentrations of 1 : 1,000, was as follows: mono-iodo saligenin, di-brom saligenin,

[1] Macht, PROC. SOC. EXP. BIOL. AND MED., 1918, **15**, 63.
[2] Macht, *J. Pharmacol. and Exp. Therap.*, 1918, **11**, 263.
[3] Hirschfelder, *J. Pharmacol. and Exp. Therap.*, 1920, **15**, 261.

mono-brom saligenin, mono-chlor saligenin, brom-iodo saligenin, chlor-brom saligenin, di-chlor saligenin, nitro saligenin, and saligenin itself. Amido saligenin produced very little anesthesia; the other compounds produced no local anesthetic effect. When applied in solid form to the tongue and mucous membranes of the mouth, di-iodo saligenin produced definite anesthesia. The di-iodo saligenin being very insoluble, however, no quantitative comparison of this compound could be made with the others.

The effect of these compounds was studied on isolated surviving muscle preparations of the uterus, intestine, urinary bladder, gall bladder, fallopian tubes, ureters, vasa deferentia, and seminal vesicles of the rat, guinea pig, rabbit and cat. While saligenin itself produced only very mild relaxation of smooth muscle, all the halogenated derivatives were much more antispasmodic than either saligenin or benzyl alcohol. This was particularly true in regard to their action on isolated intestinal and uterine preparations. Beginning with the most potent, the relative relaxant, antispasmodic efficiency of the various compounds was as follows: di-brom saligenin, mono-brom saligenin, mono-iodo saligenin, mono-chlor saligenin, di-chlor saligenin, amido saligenin, and saligenin itself. These compounds produced relaxation of smooth muscle without killing it, as proved by response to a subsequent dose of pilocarpine hydrochloride. The di-iodo saligenin, although very insoluble, produced complete relaxation of intestinal muscle in concentrations as dilute as 1 : 20,000; di-brom saligenin, in concentrations of 1 : 10,000; mono-brom saligenin, in concentrations of 1 : 8,000; mono-iodo saligenin, in concentrations of 1:6,000; and the chlor-brom saligenin in concentrations of 1 : 15,000. Nitro saligenin produced a markedly depressant effect but killed the muscle. Brom-nitro saligenin was mild in its antispasmodic action, while chlor-brom and brom-iodo saligenin acted synergistically, the effect produced by them being greater than that effected by mixtures of either mono-iodo, mono-brom, or mono-chlor saligenin. Nitroso saligenin, nitro saligenin benzoate and saligenin nitro benzoate had no effect on smooth muscle. The halogenated compounds of the series were the most interesting. They were of low toxicity, 200 to 250 mg. per kilo weight of any of them, administered by stomach tube to rabbits, producing no harmful effects. The minimal lethal doses for the bromine and iodine derivatives was 0.5 gm. per kilo weight of rabbit when given by stomach.

With the exception of the di-iodo saligenin, most of the compounds are soluble *in vitro* and physiological saline to the extent of

1 : 1,000, and even more; and, as such, were studied on intravenous injection in cats and rabbits. Intravenous injection of doses of 10 mg., and more, in cats produced a mild fall in blood pressure. Large doses, 50 mg., or more, depressed the respiration; and lethal doses (200 mg., and more) paralyzed the respiratory center. Even large doses of the drugs (200 mg. per kilo) *fed* to rabbits produced no appreciable impairment of either kidney or liver function. Because of their low toxicity, anesthetic properties, and antispasmodic effect on smooth muscle, carefully controlled clinical experiments with these compounds have been begun and are in progress.

6492

Prevention and Cure of Rickets in Rats and Antirachitic Activation of Ergosterol by Cold Quartz Mercury Lamp.*

HARRY GOLDBLATT.

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The cold quartz mercury lamp has certain definitely advantageous physical and mechanical features not possessed by other sources of ultraviolet rays.[1] One of the most striking is the fact that the heat radiation is very slight. The emission spectrum of the cold quartz mercury lamp has a limited range from about 185 mμ to about 430 mμ with its maximum intensity at about 254 mμ. By means of a balanced thermocouple and filter method of ultraviolet radiometry, Coblentz and collaborators[2, 3] measured the intensity of the radiations from a cold quartz mercury lamp. They found that the relative intensities of the cold quartz emission lines at 254 mμ, 297 mμ, and 313 mμ were 865, 14, and 45, respectively, and that over 95% of all wave lengths emitted, including the line at 313 mμ, was contained in the resonance emission line of mercury vapor at 254 mμ. By a less accurate method (a sodium photo-electric cell and filters) Hibben[1] studied the energy transmission of the grid type of cold

* Aided by a grant from the Council on Physical Therapy of The American Medical Association.

[1] Hibben, J. S., *Arch. Phys. Therapy, X-Ray, Radium*, 1931, **12**, 645, 675.

[2] Coblentz, W. W., Stair, R., and Hogue, J. M., *Bureau of Standards J. Res.*, 1931, **7**, 723.

[3] Coblentz, W. W., Stair, R., and Hogue, J. M., *Bureau of Standards J. Res.*, 1932, **8**, 759.

quartz mercury lamp and found that 74.5% of it was emitted in the region from 200 to 254 mμ. This value is probably low. The greatest intensity of the radiations of the cold quartz mercury lamp is therefore in the range 200 to 254 mμ, not usually regarded as powerfully antirachitic, although such property has been attributed[4] to some of the longer rays within this range from other artificial sources of ultraviolet radiations. Up to the present there has been no report on the antirachitic properties of the rays from this type of lamp. It seemed of interest, therefore, especially in view of the physical and mechanical advantages of this type of lamp, to investigate at least one of its possible biological properties, the ability to prevent and cure rickets in animals and to effect the antirachitic activation of ergosterol.

Prevention and cure of rickets by direct exposure to Cold Quartz Mercury Lamp. Albino rats† were fixed in the prone position on a special holder[5] with head, limbs and tail covered by rubberized cloth impermeable to ultraviolet rays. The only part of the body that was exposed to the rays was the fur covered skin of the back from the level of about the fifth dorsal vertebra to that of the middle of the sacrum. The rats were fed on rickets-producing diet No. 2965 of Steenbock and Black.[6] Exposure to the rays of the lamp was made at a distance of 5 inches from the burner. In the preventive tests the exposure ranged from 1 second to 30 minutes, and the irradiation was begun when the animals were first put on the diet. In the curative tests the exposure was begun after the animals had been fed on diet No. 2965 for 3 weeks and showed severe rickets by roentgenogram. The curative tests were begun after the preventive tests had already given a definite indication of the antirachitic properties of the lamp. Therefore the exposures in these tests ranged only from 1 second to 2 minutes.

Table I shows that at a distance of 5 inches from the lamp, irradiation of a limited portion of the fur covered body surface for 3 seconds daily was the minimum exposure which afforded complete protection against the development of rickets to rats fed on a rickets-producing diet. Table I also shows that an exposure of 10 seconds induced advanced or complete healing in a period of 2 weeks while an exposure of 5 seconds gave variable results. The minimum

[4] Sonne, C., *Strahlentherapie*, 1927, **25**, 559.

† Wistar strain, bred at the Institute of Pathology, Western Reserve University.

[5] Shohl, A. T., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 770.

[6] Steenbock, H., and Black, A., *J. Biol. Chem.*, 1925, **64**, 263.

TABLE I.

Antirachitic Effect of Direct Irradiation by a Cold Quartz Mercury Lamp.

Irradiation (seconds)	Preventive Tests Results	Curative Tests Results
1	Slight rickets	No healing
1	Very slight rickets	" "
1	" " "	——
2	" " "	No healing
2	" " "	" "
2	No rickets	——
3	" "	No healing
3	" "	" "
3	" "	——
4	" "	No healing
4	" "	" "
4	" "	——
5	" "	Very slight healing
5	" "	Slight healing
5	" "	Moderate healing
5	——	" "
10	No rickets	Healed
10	" "	"
10	——	"

curative daily irradiation to bring about complete healing in 2 weeks probably lies between 5 and 10 seconds.

In the preventive tests rats were also irradiated for 15, 20, 30, and 45 seconds, and 1, 2, 5, 10, 20, and 30 minutes (2 rats for every period) and all were completely protected. They showed no untoward effects due to the prolonged irradiation except a change in the color of the fur to a light yellow. This change did not occur in animals irradiated for less than 1 minute daily, and increased in severity with the increase in the length of the period of irradiation.

In the curative tests, rachitic rats were also irradiated for 15, 20, 30, 45, 60, and 120 seconds (2 rats for every period) and all showed complete healing at the end of 2 weeks. Advanced healing occurred in one week in rats irradiated daily for 45 seconds or longer.

Antirachitic activation of ergosterol by the radiations from a Cold Quartz Mercury Lamp. Five 10 cc. samples of a 0.2% solution of ergosterol (in olive oil) were exposed in open petri dishes 10 cm. in diameter to the radiations from a cold quartz mercury lamp at a distance of 5 inches from the burner for periods of 1, 5, 10, 20, and 30 minutes respectively. Every sample was irradiated separately and placed directly under the central portion of the burner. The solutions were exposed to the air during the irradiation. After the irradiation the solutions were diluted so that one drop (0.025 cc.) contained 0.002 mg. of irradiated ergosterol and 0.001 cc. of irradiated oil. Preventive and curative tests were then carried out on every sample. The results given in Table II show that all of the

TABLE II.
Antirachitic Activation of Ergosterol by Radiations from Cold Quartz Mercury Lamp.

Irradiation of ergosterol in oil‡ (min.)	Preventive Tests Results	Curative Tests Results
1	No rickets	Healed
1	" "	"
1	" "	"
5	" "	"
5	" "	"
5	" "	"
10	" "	"
10	" "	"
20	" "	"
20	" "	"
30	" "	"
30	" "	"
30	" "	"

‡ In both the preventive and the curative tests of the various solutions, the amount of irradiated material which every rat received daily was 0.002 mg. of irradiated ergosterol and 0.001 cc. of irradiated olive oil. The minimum protective and curative doses were not determined.

samples were active and that even of the sample that was irradiated for only 1 minute, 1 drop (0.025 cc.) daily, containing 0.002 mg. of irradiated ergosterol and 0.001 cc. of irradiated olive oil was sufficient to prevent and cure rickets in rats. The minimum preventive and curative dose of the various samples is being determined. The possible destructive effect on the vitamin D formed, due to prolonged irradiation in air and *in vacuo,* is also being investigated. A comparison of the biological properties of this lamp and of the ordinary mercury vapor quartz lamp will be reported later.

Conclusions. It has been shown that the radiations from the cold quartz mercury lamp are powerfully antirachitic. Direct irradiation for 3 seconds daily at a distance of 5 inches from the burner prevented the development of rickets in rats fed on a rickets-producing diet. Direct irradiation for 10 seconds daily for 2 weeks, at a distance of 5 inches from the burner, brought about complete healing of rickets in severely rachitic rats. Advanced healing occurred in one week in rats irradiated daily for 45 seconds or longer. Under the conditions mentioned in the text, exposure of ergosterol dissolved in olive oil to the radiations from a cold quartz mercury lamp for 1, 5, 10, 20, and 30 minutes at a distance of 5 inches from the burner resulted in the antirachitic activation of all of the solutions, of which 0.002 mg. of irradiated ergosterol and 0.001 cc. of irradiated olive oil together prevented and cured rickets in rats. The minimum protective and curative doses were not determined.

6493

Synergistic Effect of Anterior Pituitary and Male Hormone.

HOWARD T. GRABER AND RUSSELL A. COWLES.
(Introduced by John F. Norton.)

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The sex hormones of the anterior pituitary are not sex specific, but stimulate the secretion of the male as well as the female sex glands. However, for the same dose of hormone the effect on the female is far more striking than the effect on the male. In connection with other work in this laboratory, and prompted by the recent work of Funk and Harrow,[1] we carried out experiments to increase the stimulating effect of the anterior pituitary hormone on the male sex glands by simultaneous injections of male hormone.

These experiments were done on our laboratory albino rats. Eight rats of approximately equal age and weight were divided into 4 groups of 2 each; (1) control; (2) injected with anterior pituitary hormone; (3) injected with male hormone; (4) injected with both anterior pituitary and male hormone. All injections were made intramuscularly and twice daily. At autopsy the whole genital tract was exposed and the seminal vesicles and testes inspected macroscopically, and weighed.

The solution of the anterior pituitary was prepared from fresh glands and standardized by the Zondek-Aschheim technic to contain 100 mouse units in each cc. This solution was a mixture of the maturing and the luteinizing fractions as determined by previous assay on female rats.

The solution of the male hormone was prepared from fresh testes and tested on the castrated rooster for its ability to restore secondary sex characteristics. Each cc. contained approximately ½ cock unit. A cock unit is the amount of hormone necessary to cause an increase of 20% in the size of the comb and wattles in the course of 10 days. The solution of male hormone was purposely made quite dilute to preclude the possibility of any great amount of activity originating from this solution. In every case, the male hormone was itself inactive in the doses employed. In the following experiments mature and immature rats were injected twice daily over varying periods of time, and the seminal vesicles and testes examined for a degree of hypertrophy, content of secretion, ramifica-

[1] Funk, C., and Harrow, B., *Am. J. Physiol.*, 1932, **101**, 218.

tions of the glandular structure, and general appearance. All injections were run in duplicate.

In Table I, we show representative tests. The figures given represent the weights in milligrams of the seminal vesicles and testes.

TABLE I.
11 Injections in 6 Days. Autopsied on 7th Day.

Body Wt.	Immature rats, 6-7 wk. old	S.V.	Testes
65	Control	25	767
65	Control	21	737
61	.50 cc. Anterior Pituitary	38	830
66	.50 cc. Anterior Pituitary	44	1182
65	1.00 cc. Male Hormone	24	684
65	1.00 cc. Male Hormone	52	742
66	.50 cc. Ant. Pit. + 1.00 cc. Male Hormone	81	1244
58	.50 cc. Ant. Pit. + 1.00 cc. Male Hormone	64	1247
	Mature rats, 4 mo. old.		
250	Control	1078	3443
190	Control	839	2983
210	1 cc. Anterior Pituitary	1757	3064
218	1 cc. Anterior Pituitary	1575	3212
195	1 cc. Male Hormone	652	2280
225	1 cc. Male Hormone	1038	3141
210	1 cc. Ant. Pit. + 1 cc. Male Hormone	1918	3041
215	1 cc. Ant. Pit. + 1 cc. Male Hormone	1673	3228

In these experiments the weights of the seminal vesicles of the controls and the rats receiving the male hormone were practically the same. The anterior pituitary hormone alone stimulated the glands to a considerable extent, but under the influence of the combination of anterior pituitary and male hormones, the seminal vesicles were activated to a higher degree, producing greater thickness and increased secretion of the glands. In physical appearance the glands were opaque and yellowish in color, with many convolutions. Contrary to our expectations, the mature animals appeared very reactive. These results seem to indicate a sensitizing or synergistic effect between the male hormone and the anterior pituitary hormone. This effect is evident in both mature and immature rats and cannot be ascribed to purely an additive effect, since in these experiments the male hormone itself is practically inactive in the dilutions employed. This synergistic physiological effect is evident in the seminal vesicles, while in the case of the testes the results, as expected, were irregular. However, a definite hypertrophy was shown in some cases in the testes of immature rats. No attempt has been made as yet to either explain this phenomenon physiologically or to study the quantitative relationships.

6494

Possible Effect of Oil of Gaultheria in Diet of Mice Susceptible to Spontaneous Carcinoma of the Breast. I. A Suggestion.

LEONELL C. STRONG. (Introduced by C. C. Little.)

*From the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.**

The present experiment dealing with the addition of small traces of oil of gaultheria (true wintergreen oil) to the otherwise normal diet of mice, has given some interesting data. The mice used were individuals belonging to a highly inbred stock (the D strain). This stock is a branch of the dilute brown strain which has an unbroken line, mainly of brother-to-sister descent, extending over 25 years (Little and Murray). The inbreeding was initiated by Little and the stock is usually referred to as the Little Dilute Browns. My branch had passed through several individual laboratories before coming into my own. At present my dilute brown stock is in the tenth generation of pedigreed brother-to-sister matings.

Breeding females of this stock are very prone to develop spontaneous adeno-carcinoma of the breast. Murray, who has made more observations on the incidence of neoplasia in individuals of this stock than anyone else, has found more than 1300 spontaneous tumors in the past 5 years. Of all the female mice which are used as breeders, fully 80% develop carcinoma. The common infections are the cause of death of the remaining 20%. It is Murray's opinion that if all breeding females of this stock would live long enough they would all develop carcinoma of the breast.

My sub-line shows parallel findings, differing only in minor details. Whereas Murray reports 1300 spontaneous tumors, I have had only 115. This is due to the fact that individuals of other stocks in my laboratory have been better breeders and consequently have filled up most of my available cages. The incidence of spontaneous tumors in the individuals, however, is as high as it has been in the Murray derivatives of the dilute browns. If only mice that live beyond the 8 month period (when the "cancer" age is just manifesting itself) are included, then 75% of my breeding females have developed breast carcinoma. In the second place, the age at which my breeding females develop breast carcinoma is slightly later in life than those in Murray's laboratory. The mean age at which

* These experiments have been partially supported by a grant from the Josiah Macy, Jr., Foundation.

Murray's female mice develop breast carcinoma is between 10 and 11 months, whereas the value for my stock is at 12 months.

The diet on which my mice had been kept for the past 7 years consists of rolled oats, meat scrap, powdered skim milk, salt, water and Old Grist Mill dog biscuit. The first 4 items were thoroughly mixed together in the following proportion: rolled oats 90 lb., meat scrap 5 qt., powdered milk 8 qt., salt 1 lb.

The water was given in a regular 16 oz. bottle with a glass tubing drawn out to a small bore. Food and water were available to the mice at all times.

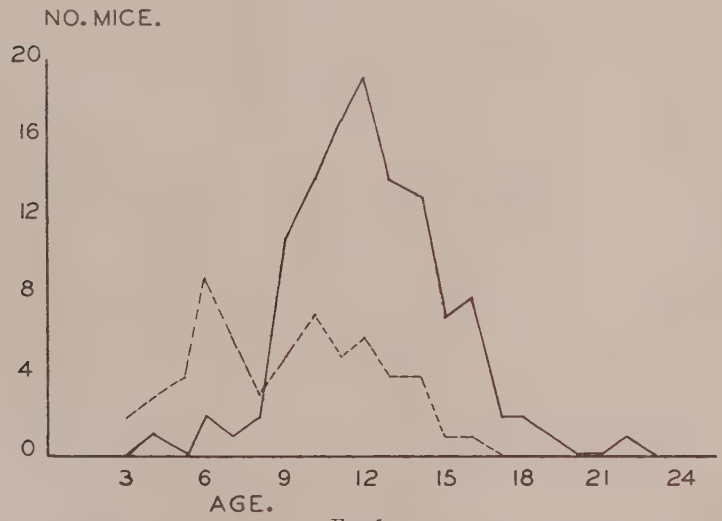

FIG. 1.

The age distribution of (1) mice developing carcinoma of the breast (solid line) and (2) the age mortality curve for all other causes of death than cancer (dash line). All mice included in this figure were kept on the normal oat meal (control) diet.

Fig. 1 shows (1) the age distribution of carcinoma and (2) the mortality curve of all other causes of death than cancer obtained with normal breeding females on the normal diet given above.

It will be noted, (1) mice of this stock go through a period of depression (as measured by the height of the mortality curve) at 6 months of age, (2) if the mice recover from this period of depression they develop carcinoma of the breast in the great majority of cases, (3) mice dying of other causes than cancer have all died by 17 months, (4) the age distribution of breast carcinoma presents a fair unimodal curve considering that only 115 cases are recorded,

and (5) the oldest mouse in this stock developed carcinoma of the breast at 22 months. The average age of mice dying of all other causes than cancer is 8.9 months.

February 26, 1932, and the subsequent 6 days, I started 45 individuals that had been used for breeders on the above rolled oat diet to which had been added small amounts of oil of gaultheria. These mice averaged 11.7 months of age. The breeding was discontinued from that time on. For 41 days, one drop of the oil was added to 10 gm. of the rolled oat diet; then for 26 days one drop of oil to 50 gm. of the rolled oat diet was given; and finally one drop to 40 gm. has been continued to the end of the experiment. No dog biscuit was given while the mice were on the oil-treated rolled oat diet.

Fig. 2 presents the data obtained.

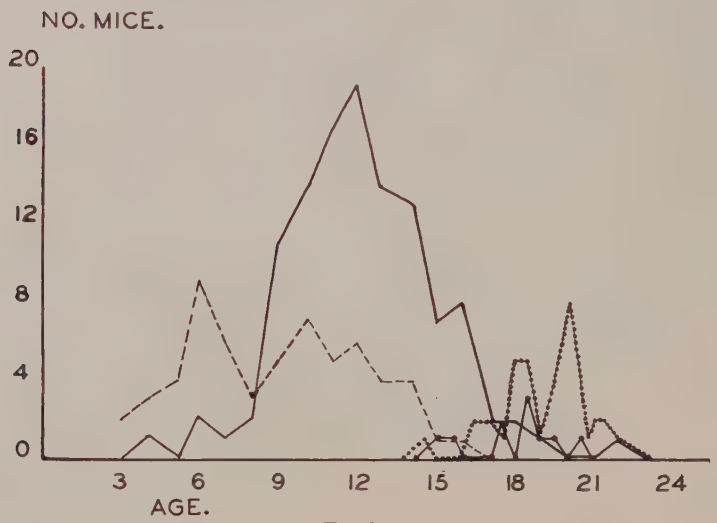

FIG. 2.

The same data as in Fig. 1 and data obtained with the oil gaultheria—rolled oat diet. The chart shows 4 classes of mice: (1) the age distribution for mice developing carcinoma of the breast on the control diet (solid line), (2) the mortality curve for mice dying from all other causes than cancer (on control oat meal diet) (dash line), (3) the age distribution for the mice that developed carcinoma of the breast on the oil gaultheria—oat meal diet (solid and ball line), and (4) the mortality curve for all other causes of death than cancer obtained with mice on the oil gaultheria—rolled oat diet (dotted line).

Ten mice of the original 45 individuals placed on the oil gaultheria diet developed spontaneous tumors. Their ages averaged 18.0 months, whereas the average for the mice on the control diet was 12.1 months. Thirty-five of the original 45 mice died of other

causes than cancer at ages beyond the time the control mice had normally developed carcinoma (average 19 months).

The experimental animals had received 2 influences other than the oil-gaultheria diet at the time of the experiment: (1) the animals were no longer used as breeders, and (2) dog biscuit had been dropped from their diet. It has been ascertained by Murray[1] that there is no difference in the cancer rate between mice that have had one litter and those that have had several. There is a real difference between virgin females and those that have been used for breeders. At no other stage in the life history of mice is there any reproductive factor that has been recognized as a disturbing element in the rate at which cancer develops. All mice used in this experiment had been used for breeders. They were placed in reserve merely to simplify their physiological behavior. Murray[2] has also shown that for the stock of mice I used the cessation of the mice from breeding does not influence either the age distribution of spontaneous carcinoma of the breast or the expectancy of life. As to discontinuing dog biscuit in the diet, several times in the past the type of dog biscuit and other food elements have been changed without any apparent effect on the cancer rate.

I realize the following limitations of the present data: (1) the number of mice used, (2) late age period for placing the mice on an experimental diet, and (3) problematical validity of the control group. These criticisms are being more carefully considered in subsequent papers in this series.

It is therefore possible that by the addition of small amounts of oil of gaultheria to the control diet, one may delay the time at which breast carcinoma would normally develop.

This finding would be quite valueless if by obtaining it the normal physiology of the individual was in any way impaired. The first effect noted was that the experimental mice ate more food than the controls. This finding verifies the work of Wiley.[3] The experimental animals did not become obese as normal mice kept in reserve from males are apt to do. Daily weights of the mice were not kept so it is impossible to determine whether the experimental animals actually lost weight. The fact that the experimental mice lived longer than the controls is presumptive evidence that they were not physiologically weakened by the special diet. The common causes

[1] Murray, W. S., unpublished data by oral communication.

[2] Murray, W. S., *Science*, 1932, **75**, 646.

[3] Wiley, H. W., Salicyl, U. S. Bur. Chem., Circ. N. 84, 1906, cited by Sollman. Manual of Pharmacology.

of death were the same as for the controls (1) pneumonia, (2) paratyphoid, (3) sarcosporidia infection, and (4) nephritis.

Whether or not any of the known facts concerning the effect of oil of gaultheria over a long period on the physiology of the organism can explain this delay in the age incidence of carcinoma is problematical. At least certain effects of this oil may be significant.

Oil of gaultheria, in common with some other salicylates,[4, 5] retards enzymic activity, especially of the digestive enzymes. According to some observers basal metabolism is elevated in patients suffering with carcinoma.[6] Is there a disturbance of basal metabolism in the earliest stages of carcinoma? Is it possible for this disturbance to be present even before cancer is obvious? Does the addition of oil of gaultheria to the diet correct this variation of basal metabolism and so exert an influence on the age incidence of cancer? These problems require more research.

These observations are being repeated on several distinct stocks of mice. At the same time the experiments call for further investigation. Is oil of gaultheria unique in this possible respect of delaying the incidence of cancer, or are other essential oils, especially those used in food flavoring, comparable? This problem is being investigated. The second problem is the effect of administration of the essential oils used in food flavoring (especially oil of gaultheria) on younger animals. It is hoped that perhaps the time at which cancer normally develops may be thus indefinitely postponed.

6495

Functional Sites in Normal and Segmentally Necrotic Renal Tubules.

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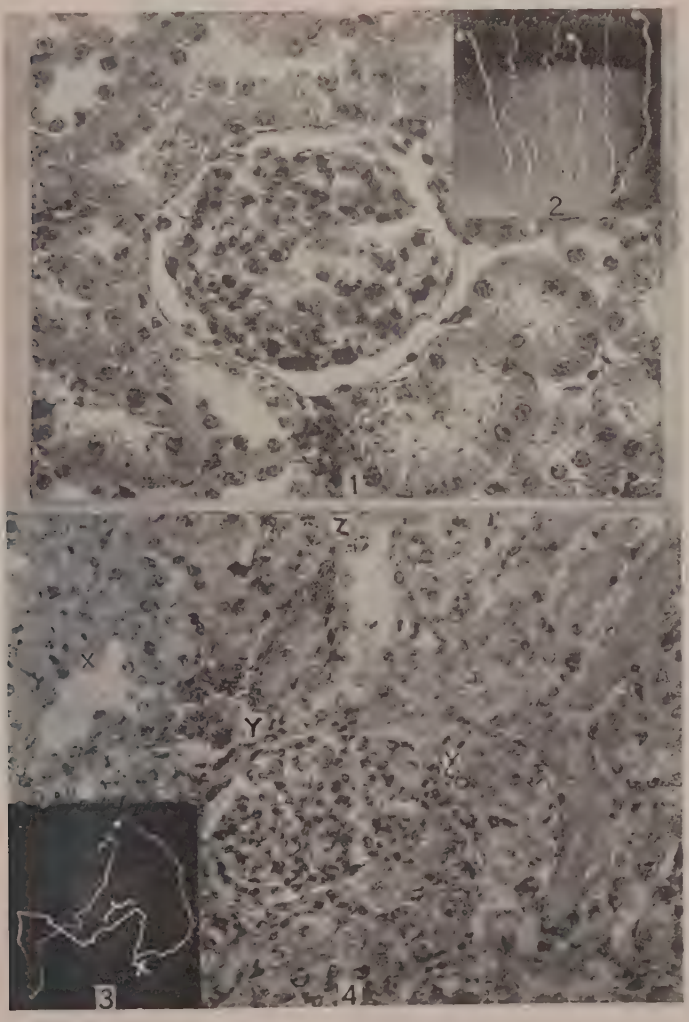
Intraperitoneal or lymph sac (frog) injections of 1% solutions of ferric ammonium citrate and sodium ferrocyanide were made in 50 rats, 10 turtles and 100 frogs. The presence of either salt or both in the kidney of these animals, depending on whether one or the

[4] Bastedo, Materia Medica, Pharmacology and Therapeutics, 3rd Ed., 1932.

[5] Cushny's Pharmacology and Therapeutics, 9th edition, 1928.

[6] Palmer, Walter W., Metabolism, Chapter 2, Vol. 3, *Nelson's Loose-Leaf Living Medicine,* November, 1926.

other or a mixture was injected, was revealed by the Prussian blue reaction. In the kidney of the rat the cellular excretion of ferric iron is sharply localized in a segment of the *proximal* convolution, 2-3 mm. long, beginning 6-8 mm. from the glomerulus (Figs. 2 and 3, portion marked by a bracket). The excretion of ferrocyanide is not similarly localized. To the extent that it may be excreted by



FIGS. 1, 2, 3, 4.

the cells of the proximal convolution, all of them appear equally active. When the 2 salts are injected as a mixture, the locus of excretion of both is the same as that stated for the excretion of the ferric salt alone. A probable explanation for this peculiarity is to appear in another publication. The accuracy of the determination of the presence and extent of this locus is the result of macerating and mounting the renal unit (Fig. 3) of the variously treated animals parallel to each other on a glass slide and studying them unstained under the microscope.

The characteristic appearance of P. blue in finely particulate form in the cells of that portion of the proximal convolution active in the excretion of iron is shown by the darker, stippled-appearing cells of the tangential section (Fig. 4, "Y"). In the kidney of the turtle and frog, the cellular excretion of both ferric iron and ferrocyanide is shared equally by the cells of the entire proximal convolution and the appearance of P. blue in such cells is very similar to that seen in the one segment of the proximal convolution of the rat.

The intracellular content of P. blue in the described sites for the excretion of iron in the kidneys of the 3 animals is increased approximately 50% by the use of urethane as an anesthetic in mildly hypnotic doses.

The cells of the ascending limb of the renal tubule of the rat and its equivalent in the turtle and frog (the proximal ⅓ of the distal convolution), those of the distal convolution proper and its duct portion in the kidney of the 3 animals are differentially resorptive as follows: water is resorbed by the cells of the ascending limb and its equivalent in the turtle and frog and solids by those of the distal convolution and its duct portion. The former is shown by the progressive luminal concentration of the iron salts (also dyes); the latter by the presence in the cells of particles of P. blue of such large size as are found elsewhere only in the lumen of this portion of the tubule (Fig. 4, black particles in section of distal convolution at left of "Z" and duct portion at its right. *Cf.*, these particles with those in section designated by "Y".)

A noteworthy support for the foregoing was found in the unexpected appearance of tubules partially necrotic in the same site of the proximal convolution for each tubule, in the kidneys of an apparently normal rat (Fig. 3, glomerulus to "X". Fig. 4, right quadrant to be compared with Fig. 1, normal renal cortex and Fig. 2, normal proximal convolutions). Since the damaged portion of the proximal convolution did not extend as far as the normal site of the excretion of iron, the appearance and presence of P. blue in various

parts of the tubule distal to the damaged portion was as already described for the normal tubule. The sharp and uniform localization of a necrotic portion within the proximal convolution indicates an antecedent functional differentiation in this portion which predisposed it to injury in this instance. Repair of the damaged epithelium was shown to have been active (Fig. 4, "X").

6496

Further Studies on Extracts Made from Holly.

RUSSELL A. WAUD.

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The author has shown that extracts made from holly have a digitalis-like effect upon the frog's heart.[1] The present work is a continuation and a more detailed study of the same problem, and an attempt to isolate the active principle in pure form. Some species of holly contain caffeine. A number of other substances have been isolated. Pancoast[2] found the berries of American holly *(Ilex opaca)* to contain pectin, albumin, 2 crystalline principles and organic salts of potassium, calcium and magnesium. The leaves of the European holly *(Ilex aquifolium Linne)* have been more carefully examined than those of any American species. They are said to contain a bitter principle, ilicin, a yellow coloring substance, ilexanthin, and a peculiar acid, ilicic acid. According to Allen's laboratory[3] Mate' which consists of the leaves of *Ilex paraguayensis* contains 0.13% caffeine.

Three species of the Ilex (Family Aquifoliaceae) were investigated, *I. opaca* (American Holly), *Ilex aquifolium* (European Holly) and *Ilex paraguayensis.* Both the fruit and the leaves of the European and the American holly were extracted but the leaves only of the South American species were available.

Preparation of Extracts. The first extracts were prepared by macerating the drug with alcohol. When used, the greater part of the alcohol was evaporated off and replaced by saline solution. Attempts were made to obtain the substance in the pure state and if

[1] Waud, R. A., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 976.

[2] Pancoast, D. P., *Am. J. Pharm.*, 1856, **28**, 314.

[3] Allens Commercial Organic Analysis, 4th ed., **6**, 642.

possible crystalline form. It was not possible, however, to obtain a crystalline substance, which, when tested pharmacologically, gave the characteristic effects. The following method, however, yielded a fairly pure form of the active substance. The drug was macerated with alcohol for 12 hours; the alcohol was then evaporated off and the residue taken up in water, placed in a separatory funnel and shaken 4 or 5 times with chloroform. Here an emulsion was formed which when allowed to evaporate left a brown powder. This was further purified by solution in alcohol and passage through charcoal.

The substance thus obtained is an amorphous powder soluble in alcohol, but insoluble in chloroform, as was proven by the following experiments: Holly berries in No. 20 powder were macerated with chloroform, filtered and allowed to evaporate spontaneously. The yellowish amorphous residue was then taken up in saline and allowed to perfuse through the frog's heart. The results were negative. Also the purified active substance was extracted with chloroform for several hours and then filtered. It was found that the active substance was not contained in the chloroform soluble portion, but remained behind. This would lead one to believe that the separation by shaking with chloroform is brought about by absorption rather than by solution. The murexide reaction is negative. After boiling with acid and subsequent neutralization the subjection of the solution to Benedict's test for sugar showed no evidence of reduction.

Attempts to isolate the active principle by the methods employed in the separation of Ouabain yielded negative results.

Pharmacodynamic Action. The pharmacology of the substance was investigated by (1) the one hour frog method for the standardization of digitalis bodies, (2) the perfused frog's heart, (3) the isolated rabbit's heart, (4) blood pressure in the rabbit, (5) the isolated uterus. The general effects of the drug were found to be very similar to those of digitalis bodies and when compared with the Canadian standard digitalis on the same frogs, 4 gm. of the crude drug was found to be equal in activity to 1 gm. of digitalis leaves.

For perfusion of the frog's heart a cannula was placed in the inferior vena cava. This was attached to a 3-way stopcock which in turn was connected with 2 aspirator bottles, one containing Ringer's solution and the other Ringer's solution plus the drug. The aortic output was determined by collecting and measuring the amount of fluid expelled from the aorta per unit of time. A perfu-

sion pressure of 45 mm. was maintained throughout the experiments. The contractions were recorded on a drum with a time marker and signal magnet for recording of the time of changing solutions.

When perfused in a ratio of 1-3000 to 1-1000 solution the frog's heart showed after a time an increase in the size of the beat, the systole being more complete while relaxation in diastole is unchanged or slightly increased; as perfusion proceeded the contraction in systole becomes more complete and the diastolic relaxation was lessened until the ventricle stopped in systolic standstill. If the drug is not washed out at this point the heart remains in the contracted condition. However, if the drug is replaced by pure Ringer's solution there is some relaxation. In the contracted condition the sinus continues to beat for several hours while the auricle is widely dilated. With the proper concentration of the drug the heart would continue to beat for hours with a considerable increase in the height of contraction and aortic output over that which existed in the first hour of perfusion before the drug was added.

If the concentration of the drug is too high the therapeutic stage is absent and the heart almost immediately goes into systolic standstill from which it does not recover.

The rabbit's heart was perfused in an apparatus similar to that designed by Eyster and Loevenhart,[4] the only important change being that the water bath was kept in motion by means of an electric windshield wiper to which 2 paddles were attached. This has the advantage over the ordinary stirrer in that there is less noise and vibration and the agitation is more complete. When perfused by the above method the rabbit's heart showed essentially the same changes as the frog's heart.

In the blood pressure experiments the rabbit was arranged for injection of the drug and the recording of blood pressure in the usual method. With comparatively large doses there were no appreciable changes in the blood pressure, nor were any other effects on the animal noted.

In the uterine experiments one horn was removed from a freshly killed guinea pig and suspended in a muscle tube filled with saline with the proper amounts of potassium, calcium, sodium bicarbonate and dextrose. The solution was maintained at 37° C. and freely oxygenated. The addition of varying amounts of the drug to the saline bath containing the uterine strip produced no evidence of oxytocic activity.

[4] Eyster, J. A. E., and Loevenhart, A. S., *J. Pharm. Exp. Ther.*, 1913, **5**, 57.

As caffeine has been found in some species of Ilex, and as it is known to have a definite effect upon the heart, the question arises as to whether this effect is due to caffeine. It does not seem possible for the following reasons: (1) The substance considered here does not respond to any of the ordinary chemical tests used for the detection of the Zanthine compounds. (2) When caffeine is extracted from some species of Ilex and perfused through the frog's heart the effect produced differs definitely from that obtained with the substance studied in this work. (3) Species of the Ilex in which no caffeine can be demonstrated show the characteristic effect.

Summary. The pharmacological action and method of preparation of a substance obtained from various species of Ilex are described. The action of the drug resembles very closely that of the digitalis bodies. It produces first an increase in the amplitude of the heart by increased relaxation and increased systolic contraction, followed by decreased relaxation in diastole with slowing and finally systolic standstill. All attempts to obtain the substance in crystalline form were unsuccessful. The possibility of the substance being caffeine is considered.

6497

Effect of Peritoneal Lavage in Acute Uremia.

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Ganter,[1] Landsberger and Gnoinski,[2] Rosenak and Siwon[3] report favorable results of peritoneal lavage in nephrectomized rabbits and dogs. The end products of nitrogen metabolism dialyze into the fluid and can be removed with it. Bliss and his coworkers[4] report that the survival period of nephrectomized dogs treated with peritoneal lavage is from 13 to 16 days as compared with the survival period of untreated dogs, from 2 to 3 days. The experiments re-

[1] Ganter, G., *Muenchen Med. Wchnschr.*, 1923, **70**, 1478.

[2] Landsberger, M., Gnoinski, H., *Compt. rend. Soc. de Biol.*, 1925, **93**, 787.

[3] Rosenak, St., Siwon, P., *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1926, **39**, 391.

[4] Bliss, S., Kastler, A. O., Nadler, S. B., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1078.

ported herein were undertaken to investigate the value of peritoneal lavage in cases of temporary acute renal insufficiency, manifested by attacks of uremia following poisoning by bichloride of mercury. A series of 15 rabbits was used. The animals were given equally large doses of bichloride of mercury (30 mg. per kilo of body weight) by stomach tube. By means of a small rubber catheter 200 cc. of an isotonic salt solution at body temperature were injected into the peritoneal cavity of the animals. This fluid was removed after 30 minutes by syphonage through the same catheter. This procedure was repeated several times at each sitting and the hemoglobin and nonprotein nitrogen content of the blood, as well as the body weight of the animals, were determined before and after each experiment. No hydremic changes in the blood could be observed. The weight of the animals decreased slightly during the course of the experiments.

Figure 1 shows the curves of the nonprotein nitrogen values in the blood of treated animal F. 22 and control animal F. 18. After each washing of the peritoneal cavity, the nonprotein nitrogen con-

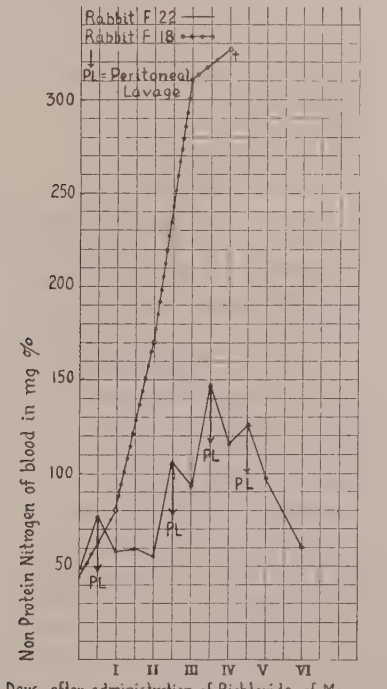

FIG. 1.

tent of the blood of rabbit F. 22 was considerably lower than before the lavage. After 5 days the kidney function of the animal was so far restored that no further washing was necessary. Control animal F. 18 died in uremic convulsions 3 days after the administration of the bichloride of mercury; the nonprotein nitrogen content of the blood was 325 mg. %. By means of peritoneal lavage rabbit F. 22 was carried over the short period of total renal insufficiency which caused the death of animal F. 18.

Table I shows the end results of our experiments.

TABLE I.

Treated Animals			Untreated Animals		
Prot. No.	Blood-NPN at end of Exp.	Remarks	Prot. No.	Blood-NPN at end of Exp.	Remarks
	mg. %			mg. %	Died days
F. 9	45	Twice periton. lav. Recovered.	F. 14	300	3
			F. 16	220	3
F. 12	235	Twice periton. lav. Died after 6 days.	F. 18	320	3
			F. 19	450	5
			F. 21	400	3
F. 15	70	Three periton. lav. Recovered.	F. 23	65	Recovered
			F. 25	80	5
F. 17	200	Three periton. lav. Recovered.	F. 26	220	4
			F. 28	280	Lavage discont. Died 6 days.
F. 20	195	Three periton. lav. Died after 7 days.			
F. 22	60	Four periton. lav. Recovered.			

Three of the 6 treated animals (F. 12, F. 17, F. 20) died with a high nonprotein nitrogen content of the blood. Rabbits F. 9, F. 15, and F. 22 recovered completely and were killed at the conclusion of the experiments in order to study the kidney changes. Post mortem examination of the animals showed no signs of peritonitis or peritoneal irritation. Microscopic examination of the kidneys of the recovered rabbits showed disappearance of the extensive tubular necrosis which had been present in the uremic animals. Post mortem examination of animals F. 12, F. 17, and F. 20 showed, in addition to the typical necrotic changes in the kidneys, a severe colitis with extensive mercurial necrosis.

Eight of the 9 control animals died from 3 to 6 days after the poisoning. In 7 animals the nonprotein nitrogen content of the blood was higher than 220 mg. % with a maximum of 450 mg. % in rabbit F. 19.

Fifty percent of animals treated by peritoneal lavage survived the period of acute uremia produced by bichloride of mercury poisoning. Only 11% of the control animals survived the experiment.

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The Effect of Ouabain on the Oxygen Consumption of Cardiac Ventricular Muscle.

JOSEPH VICTOR. (Introduced by W. O. Fenn.)

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Recently David[1] has reported that strophanthin in concentrations of 1-8 in 10^6 depresses equally both the frequency of beat and rate of oxygen consumption of the frog's auricle. Eismayer and Quincke[2] found that a low concentration (1×10^{-7}) of this substance increased the oxygen consumption of beating hearts about 20%, while high concentrations (1×10^{-5}) reduced it about 30%. But the latter concentration brought the heart to systolic standstill. The first studies on the influence of strophanthin on the oxygen consumption were those of Rhode and Ogawa,[3] who found that a heart arrested in systole by this drug consumed more oxygen than a normal beating heart. Otherwise the increase was proportional to the work done. In the reports mentioned, the drug was used in serum or Ringer's solution and the hearts were working. Clark[4] has shown the action of digitalis is dependent upon the presence of calcium ions.

In this experiment attempts were made to study the action of ouabain upon the oxygen consumption of resting cardiac muscle. The effects of this drug in sucrose and Ringer's solution were compared to see whether ions influenced the metabolic response.

Methods. The oxygen consumption measurements were done by means of the Thunberg differential volumeter modified by Fenn.[5] Each bottle had a capacity of about 15 cc. The capillary volume was about 2.5 cmm. per centimeter. The experimental bottle was fitted with 2 side-arms set at right angles to one another. The smaller one held a few drops of NaOH solution to absorb CO_2 and the larger contained the substance whose effect was to be determined. In all experiments the tissue was placed in the bottle without any solution bathing it. Four-tenths of a cc. of the reagent to be investigated were placed in the side-arm and tipped on the tissue at will. The bottles were filled with pure oxygen but were not shaken.

1 David, J. C., *J. Pharm. and Exp. Therap.*, 1930, **40**, 229.
2 Eismayer, G., and Quincke, H., *Arch. f. exp. Path. u. Pharm.*, 1930, **150**, 308.
3 Rhode, E., and Ogawa, S., *Arch. f. exp. Path. u. Pharm.*, 1912, **69**, 200.
4 Clark, A. J., *Proc. Roy. Soc. Med.*, 1912, **5**; *Therap. and Pharm.*, Sec., 181.
5 Fenn, W. O., *Am. J. Physiol.*, 1927, **80**, 327.

The cardiac ventricular muscle of *Rana pipiens* was used. The ventricle of the heart was removed along the atrioventricular groove and cut longitudinally through the apex into 2 parts of as nearly equal size as possible. After blotting with filter paper to remove adherent blood, the muscle was immediately placed in the experimental bottle. The temperature was 22.5° C. and controlled to within .01°. The Ringer's solution contained NaCl, 0.65%; KCl, 0.0075%; and $CaCl_2$, 0.01%. No buffer was used. The sucrose solution was 6.5%.

There were 12 ouabain experiments, 3 in each group, using concentrations of 10^{-5}, 3×10^{-5}, and 10^{-4} in isotonic sucrose. Three experiments were done using the drug in a concentration of 3×10^{-5} in Ringer's solution. Experiments with isotonic sucrose alone were done simultaneously. The results were consistent in every case. Control experiments on the addition of Ringer's solution were also included.

Results. The graphs in Fig. 1 represent experiments in which crystalline ouabain was added to ventricular muscle in concentrations of 10^{-4}, 3×10^{-5}, and 10^{-5} in isotonic sucrose.*

Ouabain, in a concentration of 10^{-4} in isotonic sucrose causes an immediate but gradual increase of the rate of oxygen consumption; this rate falls very slightly after about an hour, yet always remains above the original level. In a concentration of 3×10^{-5} in isotonic sucrose, the metabolic rate increases more slowly, but reaches that of the tissue bathed in 0.1 mg. ouabain per cc. The muscle bathed in a sucrose solution containing ouabain in a concentration of 10^{-5} first shows a fall in metabolic rate, probably due to the action of sucrose, but after an hour the rate increases so that 2 hours after treatment it is above normal. In these experiments the influence of ions other than those supplied by the tissue is excluded. At the end of the experiments the muscles exposed to ouabain were contracted, firm, non-irritable, and their cut surfaces curled toward one another.

* The frog dose was found to be 0.003 mg. of ouabain per gm., 0.001 mg. per gm. stopped the heart in systole in 10 minutes. Assuming the blood volume of a 30 gm. frog to be 3 cc. and concentration of drug in circulation does not change during observation, then the frog dose is 0.009 mg. for the frog or 0.003 mg. per cc. of the blood. In the frog dose the drug bathes heart muscle in a concentration of about 3×10^{-6}. The solution containing ouabain in a concentration of 10^{-5} is equivalent to a dose producing systolic arrest in the frog in 10 min. These assumptions are made because only heart muscle and red blood cells adsorb digitalis.[6]

[6] Clark, A. J., *J. Pharm. and Exp. Therap.*, 1913, **4**, 399.

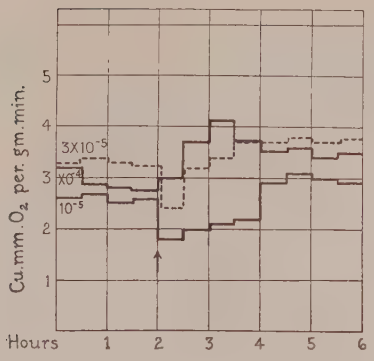

Fig. 1

Effects of ouabain in concentrations of 10^{-4}, 3×10^{-5} and 10^{-5} in isotonic sucrose solution on the oxygen consumption of resting cardiac ventricular muscle of the frog. In the 2 lower concentrations there is an immediate fall in the oxygen consumption, probably due to the action of the sucrose solution. Arrow marks the addition of the solutions.

Fig. 2

Comparison of the effects of ouabain in a concentration of 3×10^{-5} in Ringer's (solid line) and isotonic sucrose solution (dotted line). The response is greater in Ringer's solution. The time for increasing the oxygen consumption is the same in both. The arrow marks the addition of the solutions.

Ouabain in Ringer's solution in a concentration of 3×10^{-5} *produces a striking increase in respiration of almost* 100% *in contradistinction to the same amount of drug in sucrose.* (Fig. 2 and Table

TABLE I.

Conc. of Ouabain	Rate of O Consumption mm.³/gm./min. Initial	After Addition of Drug	Time for Onset of Inc. O Use, min.	% Inc. of O Cons.	Ave.
10^{-5}	2.5	3.1	140	24	
in sucrose	3.8	4.0	180	5	13
	2.7	3.0	100	11	
3×10^{-5}	3.2	3.8	30	19	
in sucrose	1.9	3.7	35	95	51
	3.3	4.6	10	39	
10^{-4}	2.7	4.2	0	56	
in sucrose	2.2	4.2	5	91	76
	3.1	5.6	0	81	
3×10^{-5}	2.3	5.0	15	117	
in Ringer's	3.0	5.9	30	96	93
	3.0	5.0	30	67	
Isotonic Sucrose	1.9	1.5		—21	
(control)	3.7	2.4		—35	—29
	2.6	1.8		—32	
Ringer's	1.9	1.7		— 9	
(control)	2.9	3.1		6	— 4
	3.0	2.7		—10	

I.) An antagonism exists between the effects of sucrose and ouabain on the oxygen consumption and tone of heart muscle. Sucrose decreases the tone and oxygen consumption of cardiac ventricular muscle[7] (Table I). In the concentration used the effects of isotonic sucrose are readily overcome by ouabain.

The relationship between the concentration of ouabain in isotonic sucrose and Ringer's solutions and the time for the metabolic response is apparent from both the graphs and the table; the more concentrated the drug, the more rapid the increase. The presence of the ions in Ringer's solution does not seem to influence the time for response, but does increase the total metabolic response. However, if the depressing effect of the sucrose solution on the oxygen consumption (average 29%) be added to the metabolic increase (average 51%), caused by the drug in a concentration of 3×10^{-5} in this solution, the difference between the total increase (80%) in this and in Ringer's solution (average 93%) is decreased.

Summary. The oxygen consumption of the resting frog's heart is about one-fourth that of the working heart.[8] Ouabain, as one of the digitaloid drugs, brings the heart to a standstill and simultaneously increases the oxygen consumption. This effect is greater in Ringer's than in sucrose solution. In certain concentrations this effect may be masked by a decrease in work, which results from cessation of beating. The time for the action of this drug on the oxygen consumption is inversely proportional to its concentration and does not seem to be affected by the presence of ions.

[7] Victor, J., *Am. J. Physiol.*, in press.

[8] Clark, A. J., and White, A. C., *J. Physiol.*, 1928, **66**, 185.

6499

Increased Stimulation of Immature Rat Ovaries by Combined Injections of Prolan and Hypophyseal Sex Hormone.*

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Experiments on the effect of combined injections of various anterior pituitary or pituitary-like hormones on the weight and changes in immature rat ovaries have given suggestive results. Fevold, Hisaw, and Leonard[1] have shown that combinations of fractions of the anterior lobe of sheep produced effects which suggested the possibility of 2 sex hormones, a follicular stimulator and a luteinizer. Leonard[2] showed that a growth hormone (phyone, van Dyke) when injected simultaneously with a sex stimulating extract of the sheep anterior pituitary, inhibited the action of the latter. More recently, Evans, Meyer and Simpson[3] have combined injections of prolan with *sex-free* growth hormone and also with *growth-free* sex hormone and have obtained activation only of their growth hormone with the production of ovaries greater than can be predicted by the additive effects of either hormone injected separately. Prolan did not react on their growth-free sex hormone which led them to conclude they were dealing with a prohormone (growth hormone) and an activator (prolan).

We here present data concerning the effect of single and combined injections of prolan with the sex hypophyseal hormone of sheep glands prepared by the pyridine method.[1] Only the water soluble fraction of the pyridine extract was used. The prolan was prepared by the alcoholic precipitation method as described by Zondek. In a litter of at least 3 immature rats between the ages of 21-24 days, one received prolan in doses sufficient to produce at least several corpora lutea in the ovaries in 5 days, one received a known amount of the hypophyseal extract, and the other a combination of the two. The volume of injected fluid was the same in all cases,

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† National Research Fellow, Biological Sciences.

Sheep glands were furnished through the courtesy of Dr. O. P. Kamm, Parke, Davis & Company.

1 Fevold, Hisaw and Leonard, *Am. J. Physiol.*, 1931, **97**, 291.

2 Leonard, *Am. J. Physiol.*, 1931, **98**, 406.

3 Evans, Meyer and Simpson, *Am. J. Physiol.*, 1932, **100**, 141.

and similar results were obtained whether the combined substances were mixed before injection or injected separately. *In all experiments, the absolute increase in the weight of the ovaries produced by the combined injections was greater than could be predicted by the sum of the increased weight produced by each extract separately.* The ovaries were increased from 20% to 466% over the expected increase with an average of 142%. Only littermate animals were compared, the total number used being 53 in 10 litters. Histological differences in the ovaries of these animals cannot be reported at this time but examination under the binocular did not reveal any striking difference except size.

While results from combined injections are not explainable at present, empirically they may be of use when great ovarian stimulation is to be desired by the administration of sex stimulating hypophyseal hormones. The explanation cannot be on the theoretical basis proposed by Evans, Meyer and Simpson because (1) that the sex hormone could not in any way be activated by prolan, (2) that the hypophyseal extracts in these experiments exhibited no growth response when tested on dwarf mice, which are extremely sensitive to growth hormone. Neither could it stimulate growth in hypophysectomized rats. However, sheep glands were used as a source of hormone, while the other group of workers used beef glands, and the preparations of extracts were quite different. Species differences in threshold of response of the reaction of the receptor organ also may account for this disagreement in results. That prolan can act at its maximum efficiency only in the presence of circulating hypophyseal hormones is demonstrated from the apparent ineffectiveness of prolan on the hypophysectomized animal (Reichert *et al.*[4]) and White and Leonard (1932 in press).

Pyridine extract of dried human placenta acts like prolan when combined with the gland extract to give this *super-stimulating* effect. This is not surprising since unpublished data shows that the pituitary-like sex hormone of the placenta is similar to prolan.

[4] Reichert *et al.*, *Am. J. Physiol.*, 1932, **100**, 157.

6500

Destruction of Reducing Sugars by Resting Bacterium coli.*

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From the Buffalo General Hospital and the University of Buffalo.

The study of carbohydrates by the destructive action of yeast and bacteria has been carried out along different lines.[1] One of us[2] has caused differential sugar destruction by exposing test solutions to suspensions of resting yeast cells and then to bacterial growth. The present investigation was undertaken to study quantitatively the destruction of various sugars by resting bacteria grown upon sugar-free medium and medium enriched with various sugars.

A typical strain of *B. coli* (No. 4348 from the American Type Culture Collection) was grown upon a medium prepared as follows: Beef juice broth rendered sugar-free by the usual incubation with *B. coli,* filtered and adjusted to pH 7.6, 1.5% agar added. This mixture, alone or enriched with sugars, (0.5%) was slanted in culture tubes 1 inch in diameter. The slants were heavily seeded so that the entire surface was covered with organisms, and allowed to incubate at 37.5° C. for 18 hours. The growth was then removed with a small volume of physiological saline and the "resting" organisms washed at least twice with saline by centrifuging. About 0.45 to 0.55 cc. of packed organisms were suspended in 4 cc. of a solution containing 2.85% Na_2HPO_4 and 1.005% NaH_2PO_4. The suspension was brought to a temperature of 37.5° C. in a water bath, 2 cc. of an aqueous solution of carbohydrate† at the same temperature added, the contents of the tubes thoroughly mixed and incubation carried out for 10 minutes in the water bath. The tubes were immersed in melting ice until they were thoroughly chilled

* This material formed part of a paper read before the Syracuse meeting of the Western New York Branch of the Society for Experimental Biology and Medicine in May, 1932.

[1] Peters, J. P., and Van Slyke, D. D., Quantitative Clinical Chemistry, 1932, **2**, 478. Baltimore.

[2] Hubbard, R. S., and Allison, C. B., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 408; *Clifton Med. Bull.*, 1928, **14**, 35; Hubbard, R. S., and Deegan, J. K., *J. Biol. Chem.*, 1928, **77**, lvii; 1930, **86**, 575; Hubbard, R. S., and Wilson, D. C., *Clifton Med. Bull.*, 1930, **17**, 57; Hubbard, R. S., and Kingsbury, M., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 93.

† The amount of sugar in this solution was usually a little greater than was destroyed by the organisms as shown by preliminary experiments. It varied from about 0.003% to 0.6% for the different carbohydrates studied.

and the organisms thrown down by high speed centrifuging. The clear supernatant liquid was then decanted and its reducing power determined by the method of Folin and Wu, using as standards, solutions of the appropriate sugars in the phosphate solution. There was a slight blank value which varied between 0.5 and 1.5 mg. of glucose per 100 cc. This was determined in each test upon suspensions of organisms containing no sugar.

Among the carbohydrates studied (glucose, mannose, levulose, galactose, maltose, xylose, and arabinose) there were no significant differences in destructive ability of the organisms whether grown on sugar-free medium or medium enriched with glucose or mannose. In each case glucose and mannose were destroyed in large and approximately equal amounts (9 mg.). Levulose, galactose, and maltose were destroyed in much smaller quantities, 0.6, 0.2, and 0.2 mg., respectively. No demonstrable destruction of lactose, xylose, or arabinose was obtained.

When the organisms were grown upon medium enriched with levulose, maltose, xylose, or arabinose, their ability to destroy the corresponding sugar became very marked. No change was noted in their ability to attack the other sugars. When, however, either lactose or galactose were used to enrich the medium, the resulting organisms developed a reciprocal ability to destroy both sugars. Again no change was noted in their ability to destroy other sugars.

A number of rather interesting inferences may be drawn from the results. In spite of the fact that all common sugars are readily attacked by *B. coli* during growth, the ability to destroy some of them is usually latent or is present only to a limited extent in the organisms themselves. When exposed to these sugars for only a comparatively short period of time, the ability to destroy them is markedly increased. In some instances a new ability to attack certain sugars seemed to develop under these conditions, but it is possible that the sugars in question—arabinose, xylose, and lactose—may have been destroyed in the control experiments in amounts too small to be demonstrated by the technique used. It is practically impossible to believe that the results are due to the development of a variant of the organism in the presence of an unusual sugar in the culture medium, for the effect of the resting organisms upon sugars other than the specific one was in general not influenced by these variations in the methods of cultivation. We believe that the results can be most readily interpreted by assuming that *B. coli* is an organism with great ability to adapt itself to changes in the type of carbohydrate furnished it and that the ability to destroy such

carbohydrates becomes a definite property of the particular organism which has once developed it.

The actions of *B. coli* independent of the method of cultivation are also of interest. The organism behaved toward glucose and mannose in exactly the same manner, an observation which, as far as the authors know, has not been described in biological studies. Others have described a similar relationship between glucose and levulose based on experiments with yeast. A consideration of the structural formulas of these 3 sugars justifies interesting speculation regarding the relationships between the structure of carbohydrates and the mode of enzyme action.

In one instance the results seem to indicate the general method in which the sugar was destroyed. Organisms grown upon a medium containing either galactose or lactose developed an equal ability for destroying both sugars. It seems probable, therefore, that the attack was upon the whole lactose molecule rather than by means of hydrolysis of the sugar.

These results of quantitative studies of the destruction of sugars by resting *B. coli* seem to indicate that this method of study may be of considerable value in attacking problems in the physiology of bacteria.

6501

Quantitative Variations in Destruction of Glucose by Resting Bacterium coli under Different Experimental Conditions.*

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In spite of the extent to which masses of yeast cells have been used in determining the true glucose content of blood, quantitative data upon the various factors which may affect the reaction are meagre. There seems to be even less available information upon the destruction of glucose by bacterial cells. The present report concerns quantitative studies of some of the factors involved in the destruction of glucose by "resting" *B. coli.* The general methods were those described in the preceding paper.

* This material formed part of a paper read before the Syracuse meeting of the Western New York Branch of the Society for Experimental Biology and Medicine in May, 1932.

The effect of acid and alkali upon the destruction of glucose was first investigated. No destruction was demonstrated if glucose was added to organisms suspended in 0.01 N hydrochloric acid, and very little if 0.001 N acid was used. In distilled water some destruction took place, but in 0.001 N sodium hydroxide this was more pronounced. If the strength of alkali was increased the amounts destroyed lessened, and in 0.02 N alkali no destruction of glucose could be demonstrated. It seemed clear, therefore, that a buffered solution of moderate alkalinity would furnish suitable conditions for carrying out studies of the decomposition of sugars and, accordingly, the alkaline buffered phosphate solution described in the preceding paper was adopted. It is the same one used in earlier work upon the destruction of glucose in blood filtrates by incubation with the *B. coli.*

The effect of varying the number of organisms and the concentration of sugar was next investigated. It could easily be shown that the amount of glucose destroyed paralleled quite closely the concentration of the bacterial suspension, but it was necessary to control conditions carefully to demonstrate the effect of varying

TABLE I.

Removal of glucose from 6 cc. of phosphate solution by exposure to a heavy suspension of *B. coli* for 10 minutes at 37.5°.

Exp.† No.	Glucose in solution incubated	Glucose found after incubation	Glucose removed by organisms‡	
			difference	Amt.
	%	%	%	mg.
1	0.100	0.001	0.100	6.0 (+)
	0.150	0.012	0.137	8.2
	0.200	0.062	0.137	8.2
	0.250	0.106	0.143	8.6
2	0.100	0.001	0.100	6.0 (+)
	0.150	0.021	0.138	8.3
	0.200	0.066	0.133	8.0
	0.300	0.150	0.149	8.9
	0.400	0.280	0.119	7.1

† In each experiment the same concentration of organisms (approximately 0.5 cc. packed cells) was present in every test.

‡ In all tables results have been corrected for the blank, (+) used when all the glucose present was destroyed, and under "difference" the difference between the % present at the beginning of the experiment and that found in the supernatant fluid after incubation and removal of the organisms indicated.

All standard and unknown solutions contained the same concentration of phosphate.

It is probable that there was a high % of error when the initial sugar content was large, because it was then necessary to dilute the solution greatly (4 to 10 times) before the colorimetric determination was carried out and the effect of any error was, therefore, greatly increased. The variations in the last 2 columns are all smaller than would be occasioned by an error of 5% in the determination of glucose.

concentrations of glucose in the presence of a constant concentration of bacteria. A large volume of packed washed organisms was suspended as evenly as possible in salt solution, and equal volumes pipetted into centrifuge tubes. These organisms were then centrifuged, the supernatant fluid decanted and the organisms resuspended in buffer solution containing various concentrations of glucose. The 2 protocols in Table I show how closely results can be duplicated when conditions are carefully controlled. The reaction is a definitely quantitative one, and when a large concentration of organisms is used the amount of glucose present does not measurably affect the amount of it which is destroyed.

Since the results obtained under controlled conditions were so concordant, other factors were varied and the effect of the variations determined. Table II shows the results when different incubation periods were used. Very short periods were not investigated because it was felt that the accuracy of the results would be very low, since some destruction of glucose undoubtedly takes place when the organisms are first immersed in the cooling bath. Long incubation was not carried out because we wished to avoid multiplication of the organisms. The results in the table show that the reaction appeared to proceed more rapidly during the first part than during the later stages of incubation. It seems probable that the apparent retardation of the reaction was not due to exhaustion of the organisms, for when bacteria which had been used in one experiment were washed with salt solution, placed on ice over night and used again, they showed no demonstrable change in their ability to destroy glucose. It seems more probable that the retardation shown was due to an accumulation of waste products either in the organisms themselves or in the medium in which they were suspended.

The effect of temperature was then investigated. The results are

TABLE II.

Effect of variations in the time of incubation at 37.5° upon removal of glucose.

Incubation	Packed Organisms	Glucose in solution incubated	Glucose removed by 0.5 cc. organisms	
			difference	Amt.
min.	cc.	%	%	mg.
5	0.45	0.050	0.050	3 (+)
10	0.47	0.050	0.050	3 (+)
5	0.49	0.100	0.083	5.0
10	0.43	0.100	0.100	6 (+)
20	0.47	0.100	0.100	6 (+)
5	0.45	0.200	0.085	5.1
10	0.45	0.200	0.141	8.5
20	0.43	0.200	0.206	12.4

shown in Table III. It is evident that the speed of the reaction increases as the temperature increases and, therefore, it is almost certain that the removal of glucose from phosphate solutions by *B. coli* is not an adsorption phenomenon.

TABLE III.
Effect of variations in temperature upon the destruction of glucose.

Temperature	Incubation	Packed organisms	Glucose in solution incubated	Glucose removed by 0.5 cc. organisms	
				difference	Amt.
	min.	cc.	%	%	mg.
27.5° C.	5	0.45	0.050	0.050	3 (+)
	10	0.38	0.050	0.050	3 (+)
	5	0.40	0.100	0.075	4.5
	10	0.40	0.100	0.107	6.4
	20	0.40	0.100	0.100	6 (+)
	5	0.50	0.200	0.061	3.7
	10	0.40	0.200	0.113	6.4
	20	0.36	0.200	0.170	10.2
47.5° C.	5	0.48	0.050	0.050	3 (+)
	10	0.40	0.050	0.050	3 (+)
	5	0.45	0.100	0.097	5.8
	10	0.42	0.100	0.100	6 (+)
	20	0.42	0.100	0.100	6 (+)
	5	0.50	0.200	0.104	6.2
	10	0.42	0.200	0.229	13.7
	20	0.40	0.200	0.200	12 (+)

The experiments reported show that the destruction of glucose by masses of *B. coli* is a reaction or series of reactions which can be studied quantitatively and the physical chemical characteristics of which can, at least to some extent, be determined. When a large concentration of organisms was used and the reaction proceeded rapidly, the amount of sugar destroyed was a function of the number of cells, but not of the concentration of glucose present. The reaction velocity was not linear, for relatively more glucose was destroyed in 5 than in 20 minutes. The enzyme responsible for the destruction could not be washed out of the cells readily and apparently was not used up during the reaction, for the same organisms when used in successive tests showed no demonstrable change in their efficiency. The rate of the removal of glucose varied directly with the temperature and, therefore, at least one of the reactions involved could not be classed as an adsorption phenomenon.